HISTOPATHOLOGICAL STUDY AND IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN LUNG TUMOURS - A HOSPITAL BASED STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI."

By

REG NO: BN0119009

Dissertation

Submitted to the

KLE Academy of Higher Education and Research
Belagavi, Karnataka
In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE
IN
PATHOLOGY

DEPARTMENT OF PATHOLOGY
J. N. MEDICAL COLLEGE, BELAGAVI
KARNATAKA

APRIL – 2022

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

BELAGAVI, KARNATAKA

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This is to certify that the dissertation entitled **"HISTOPATHOLOGICAL STUDY AND IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN LUNG TUMOURS - A HOSPITAL BASED STUDY AT KLES DR.PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI"** is a bonafide research work done by **REG NO: BN0119009**.

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Ref No: MDC/PG/ Date: 17-11-2021

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LIST OF ABBREVIATIONS USED

EGFR	-	Epidermal Growth Factor Receptor
TKIs	-	Tyrosine Kinase Inhibitors
IHC	-	Immunohistochemistry
'P' value	-	Probability value
H-score	-	Histo-score
SCC	-	Squamous Cell Carcinoma
SCLCs	-	Small Cell Lung Cancers
NSCLCs	-	Non - Small Cell Lung Cancers
IUL	-	Intra-Uterine Life
FISH	-	Fluorescence In-Situ Hybridisation
WHO	-	World Health Organization
SD	-	Standard Deviation

ABSTRACT

HISTOPATHOLOGICAL STUDY AND IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN LUNG TUMOURS - A HOSPITAL BASED STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI.

Background & Objectives:

Currently, lung cancer is the commonest etiology for cancer mortality in world. In the present-day scenario, it is one of the major cancers diagnosed frequently. Lung cancer has proven to kill more patients compared to those suffering from breast, colon and prostate cancers combined. Statistically, for 1.8 million deaths reported due to lung cancer, out of estimated 2.1 million new cases formed in year 2018, lung cancer stands out to be a major cause of mortality worldwide.

As most of the lung cancers are in advanced stage of disease at the time of diagnosis, screening attempts have to be developed in future so that the cancer can be identified for the better outcome and prognosis. Adenocarcinoma is now the most common form of cancer compared to squamous cell carcinoma and small cell carcinoma of the lung. Till a decade ago, EGFR mutation analysis by direct sequencing was studied and was a dependable method to predict response to TKIs. Further, it was studied that both EGFR mutation and gene amplification status were essential to know which tumours will respond to TKIs.

In 2015, an Indian study was conducted for evaluation of EGFR expression by IHC method, which concluded that EGFR expression is associated with a 6

month survival post chemotherapy. It was also concluded that overexpression (H-score>200) was associated with poor prognosis.

The arrival of successful molecular targeted therapies which are directed at specific cell types as well as subtypes, has augmented the need for a more particular subtyping and classification of these cancers. Hence, the present study is being conducted to study histopathological spectrum of lung cancer types and EGFR expression by IHC.

Materials and Methods:

A total of 40 cases of lung tumours were included from January 2019 to December 2020. Clinical details and gross findings were obtained from medical records and grossing notes using a structured proforma. Paraffin embedded blocks were archived and histological findings & IHC expression of EGFR were analysed. Statistical analysis was done using chi square test and 'p' value of less than 0.05 was considered statistically significant.

Results:

Peak incidence of 40% of lung tumours was noted in the age group of 51-60 years, with a clear male preponderance. 65% were smokers, 52.50% had mixed diet intake and 15% were positive for family history of lung cancer. 50% of tumours in the study were located in the upper lobe of right lung. 52.50% were adenocarcinomas and 32.50% were squamous cell carcinomas with a single case of lepidic variant of adenocarcinoma and keratinizing variant of SCC each. 85% cases showed positive staining intensity for EGFR, among which 10% were weak positive, 35% were intermediate and 40% were strong positive. 72.50% cases were EGFR IHC positive

by H-score method. 87.50% had cough as the most common symptom, 62.50% had dyspnoea, 62.50 % showed loss of appetite, 67.50% presented with weight loss and 45% had hemoptysis.

Conclusion: Increasing age and types of lung tumours like squamous cell carcinomas, adenocarcinomas and their variants, showed a significant association with intensity of EGFR staining. A significant association between H-score and types of lung tumours was established in this study. Thus, the expression of EGFR protein can be used as a marker for targeted therapies in patients suffering from these lung tumours.

Keywords: Lung cancer, EGFR, immunohistochemical

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

LIST OF CHARTS

LIST OF PHOTOMICROGRAPHS

INTRODUCTION

Currently, the most common cause of cancer related deaths globally is lung cancer. In the present-day scenario, it is one of the major cancers diagnosed frequently. Lung cancer has proven to kill more patients compared to those suffering from breast, colon and prostate cancers combined. Statistically, for 1.8 million deaths reported due to lung cancer, out of estimated 2.1 million new cases formed in year 2018, lung cancer stands out to be a major cause of mortality worldwide.[1]

As most of the lung cancers are in progressive phase of disease in the period of time of diagnosis, screening attempts have to be developed in future so that the cancer can be identified for the better outcome and prognosis. Adenocarcinoma is at present the commonest type of cancer compared to squamous cell carcinoma and small cell carcinoma of the lung.[2]

Growth and metastasis of lung cancers include EGFR dependent activations of Ras/MAPK and PI3K/AKT. PI3K/AKT is a pre-proliferative signaling pathway that enhances cell multiplication and then weakens apoptotic mechanisms in SCLC and NSCLC.[3]

Till a decade ago, EGFR mutation analysis by direct sequencing was studied and was a dependable method to predict response to TKIs. Further, it was studied that both EGFR mutation and gene amplification status were essential to know which tumours will respond to TKIs.[4]

In 2015, an Indian study was conducted for evaluation of EGFR expression by IHC method, which concluded that EGFR expression is associated with a 6 month survival post chemotherapy. It was also concluded that overexpression (H-score>200) was associated with poor prognosis.[5]

The arrival of successful molecular targeted therapies which are directed at specific cell types as well as subtypes, has augmented the need for a more particular subtyping and classification of these cancers.

Hence, the present study is being conducted to study histopathological spectrum of lung cancer types and EGFR expression by IHC.

AIMS AND OBJECTIVES

1) To study the expression of Epidermal Growth Factor Receptor in lung tumours.

2) To study histopathological types of various tumours of lung.

REVIEW OF LITERATURE

Embryology of lungs[6]

The first phase is embryologic phase which occurs at 0 to 7 weeks of IUL and gives rise to lung buds and major pulmonary arteries.

The second phase is pseudoglandular phase that takes place at 5 to 17 weeks of IUL and leads to development of airways and the blood vessels at the acinar level.

The third phase is the canalicular phase occurring at 17 to 27 weeks of IUL that helps in forming respiratory airways and thinning of blood-gas barrier.

The fourth phase is the alveolar phase, also called as saccular phase which occurs at 28 weeks to term of IUL, finally forms the first alveolar appearance in lungs.

The primordial lungs originate as the ventral buds of the foregut that extend into the primitive thoracic mesenchyme. Derived from the mesenchyme are the cartilages of bronchi, smooth muscle and the connective tissues which environ these dichotomously diverging buds.

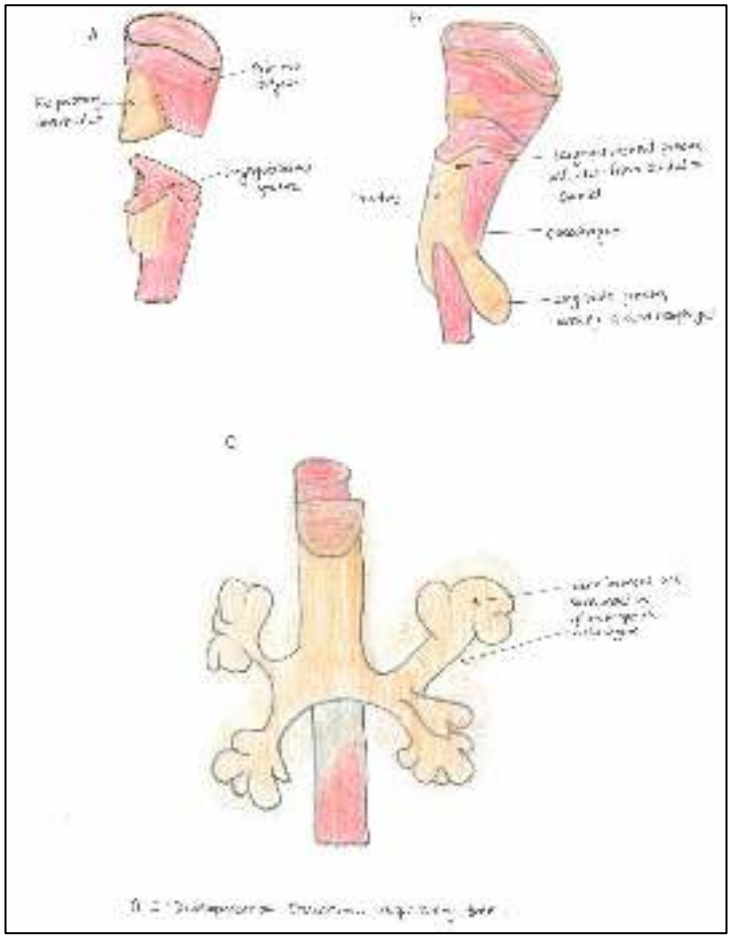


Fig.1.Development of endodermal respiratory tree

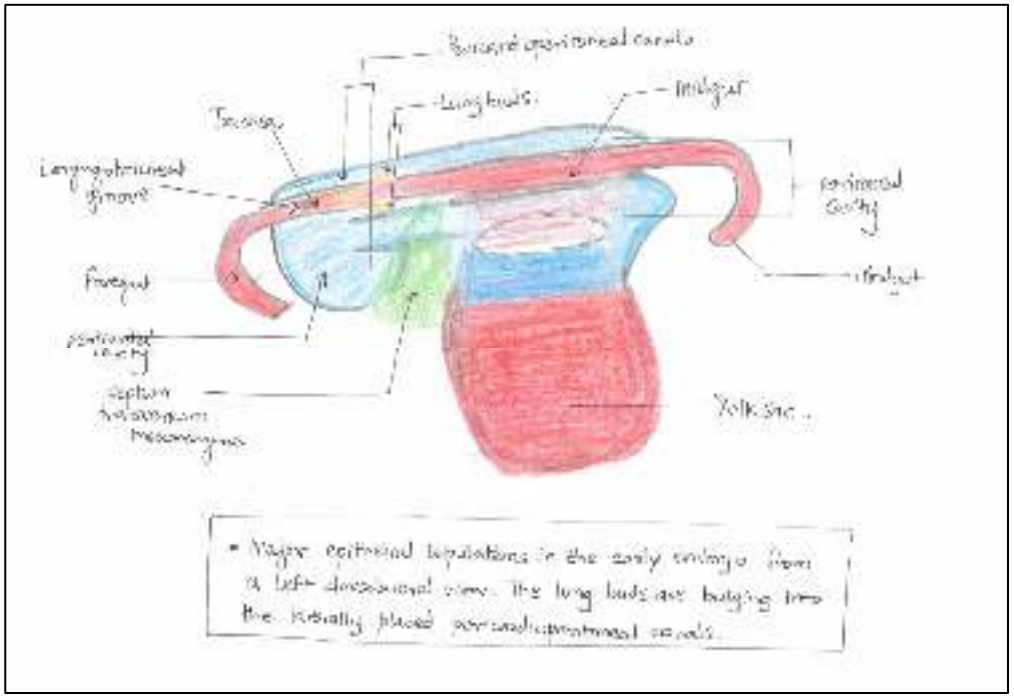


Fig.2.Development of lung buds

Functions and Anatomy of Lungs[7]

The lungs are necessary organs of respiration. They are responsible for exchange of gases such as oxygen and carbon dioxide. They also function as the first line of defence by acting as a mucous barrier and have a prime role in cough reflex mechanisms.

They are placed in either sides of heart inhabiting most of the thoracic cavity. Grossly, the external surface of lungs is smooth and shiny. The usual weight of lungs in adult is 625 g and 565 g for right lung and left lung respectively. The features on pulmonary surface for each lung include an apex, base, three borders and two surfaces.

The right lung is divided into three lobes by oblique(major) and horizontal (minor) fissure named as superior, middle and inferior lobes.

The left lung is divided into two lobes-superior and inferior by a single oblique fissure.

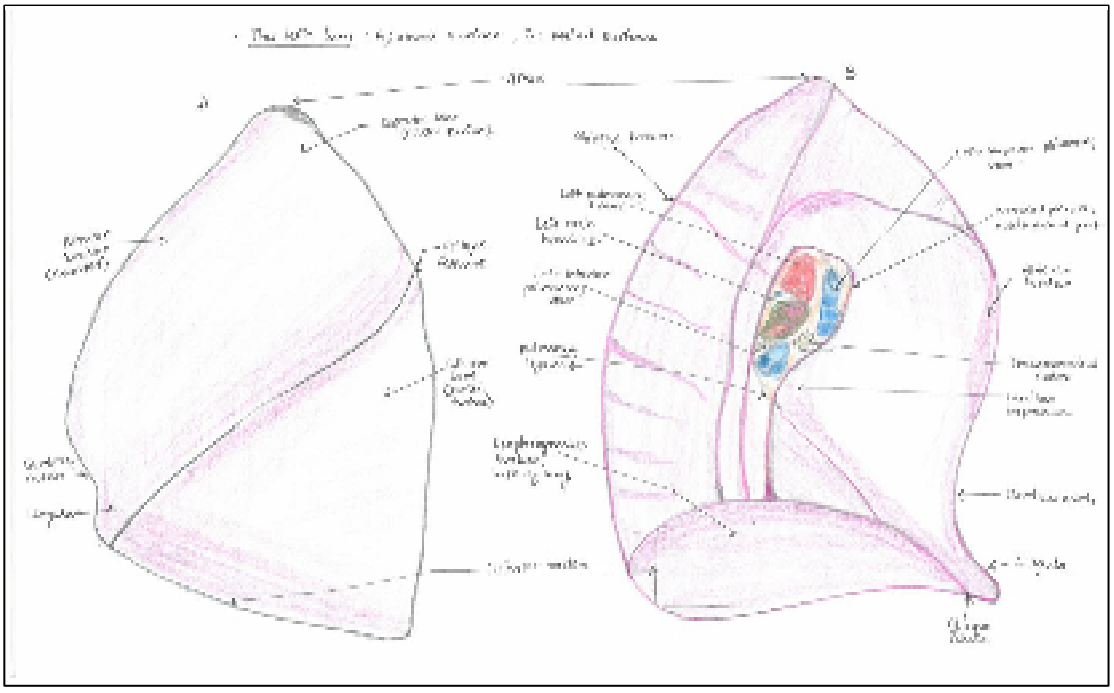


Fig.3.The left lung: surface anatomy

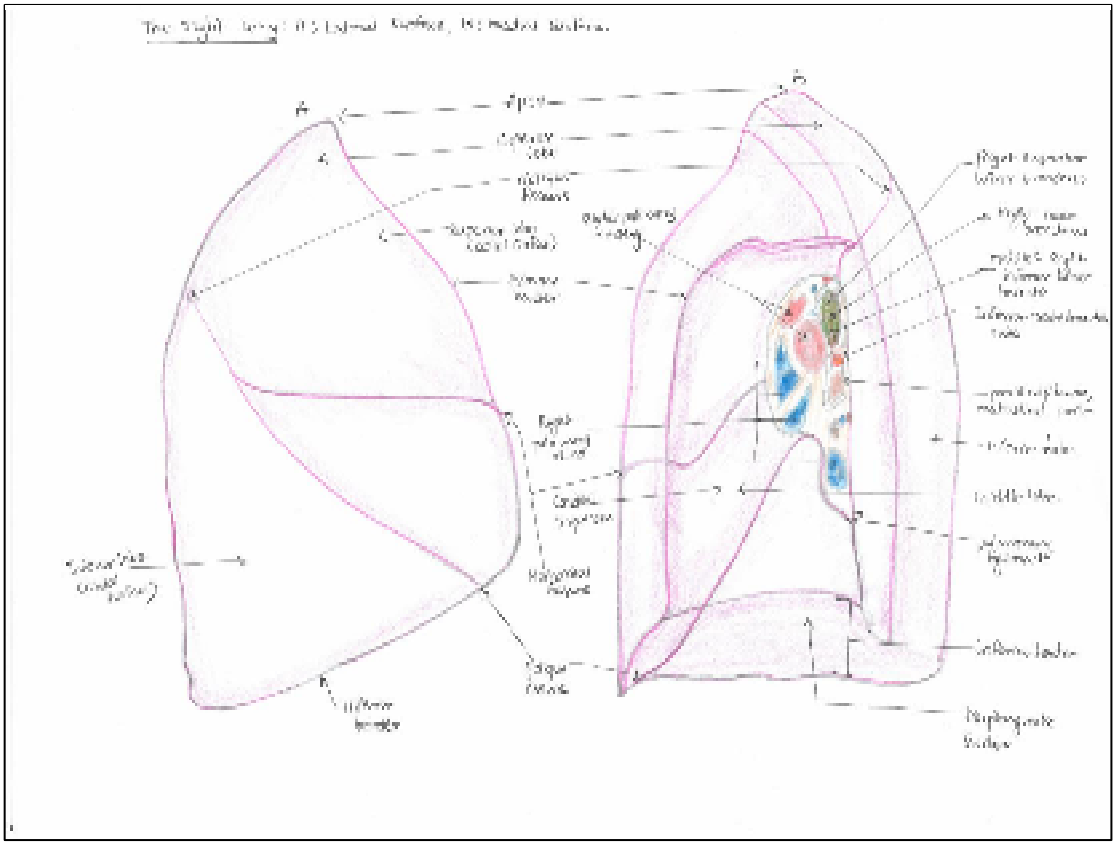


Fig 4.The right lung: surface anatomy

Trachea is a 10 to 11 cm hollow tube comprised of cartilage and fibromuscular membrane. The right and left principal bronchi (primary) divide into lobar bronchi (secondary). The primary branches of lobar bronchi are the segmental bronchi (tertiary) which supply air to bronchopulmonary segments.

The segmental bronchi (tertiary), after several sequential divisions, divide to give terminal bronchi, lobular bronchioles, terminal bronchioles, respiratory bronchioles, alveolar ducts and eventually alveoli. Starting from the trachea, there are about 20 generations in a normal human lung that stretch till the respiratory bronchioles.

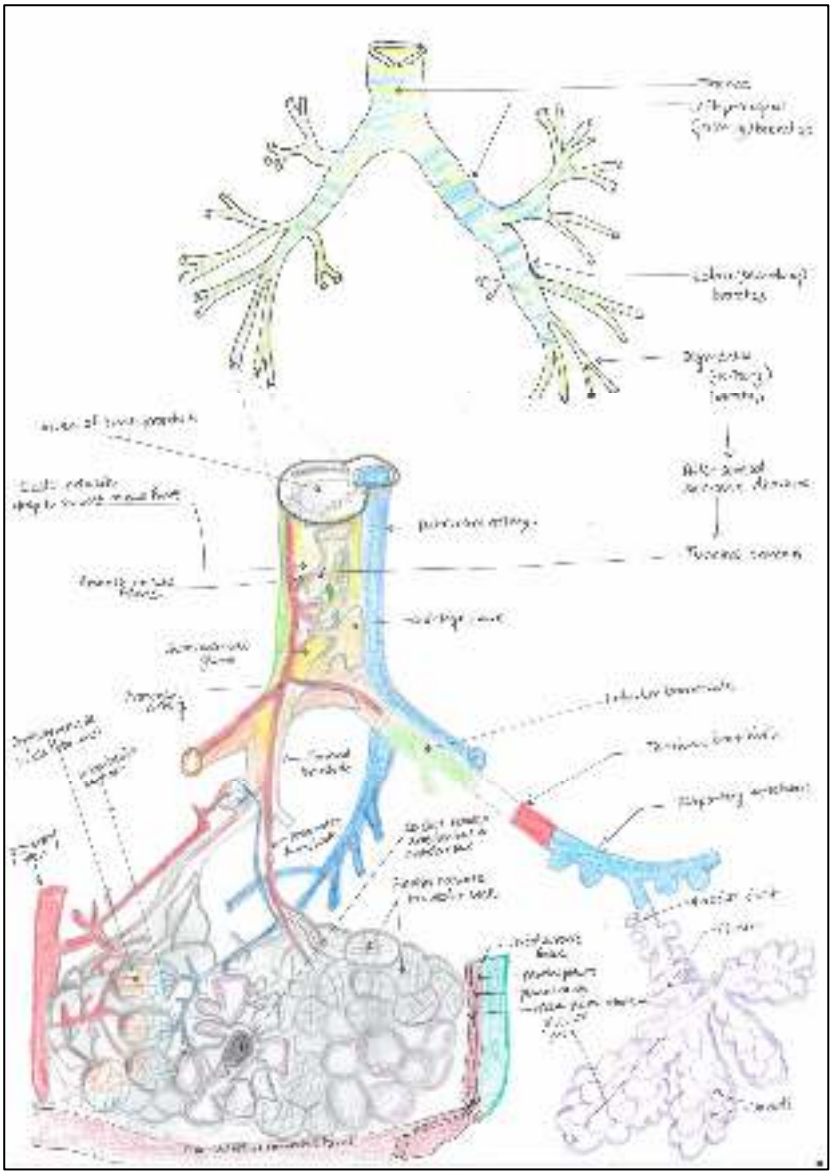


Fig.5.Trachea and its divisions

The bronchopulmonary segments do not have their own pleural investments and are constituted based on their respective segmental bronchi.

The various lobes of lung consist of the following bronchopulmonary segments:

The right upper lobe - apical, posterior and anterior.

The right middle lobe - lateral and medial.

The right lower lobe - superior, medial basal, anterior basal, lateral basal and posterior basal.

The left upper lobe - apical, posterior and anterior.

The lingula - superior and inferior.

The left lower lobe - superior, antero-medial basal, lateral basal, posterior basal.

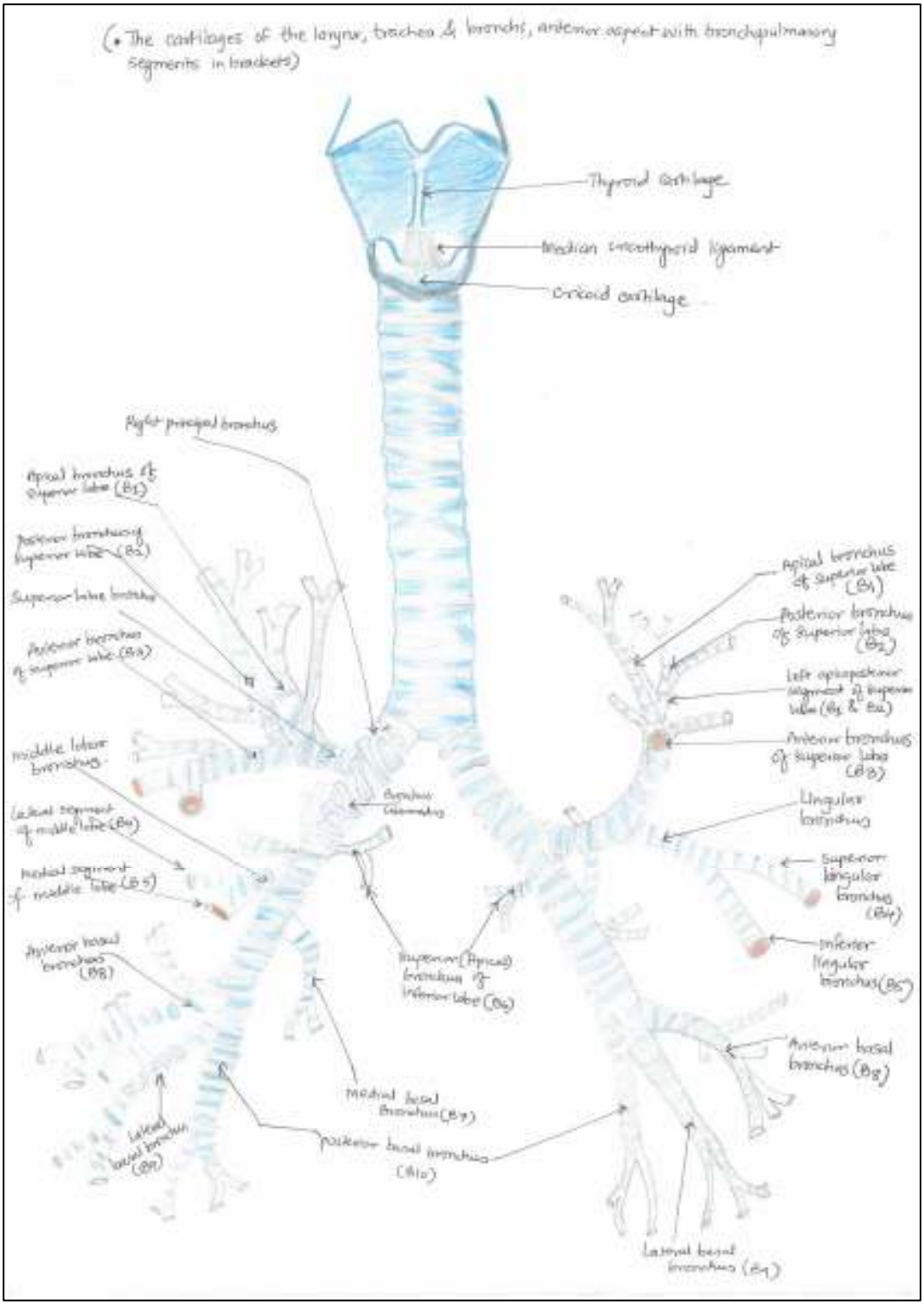


Fig 6. Tracheal divisions and associated bronchopulmonary segments

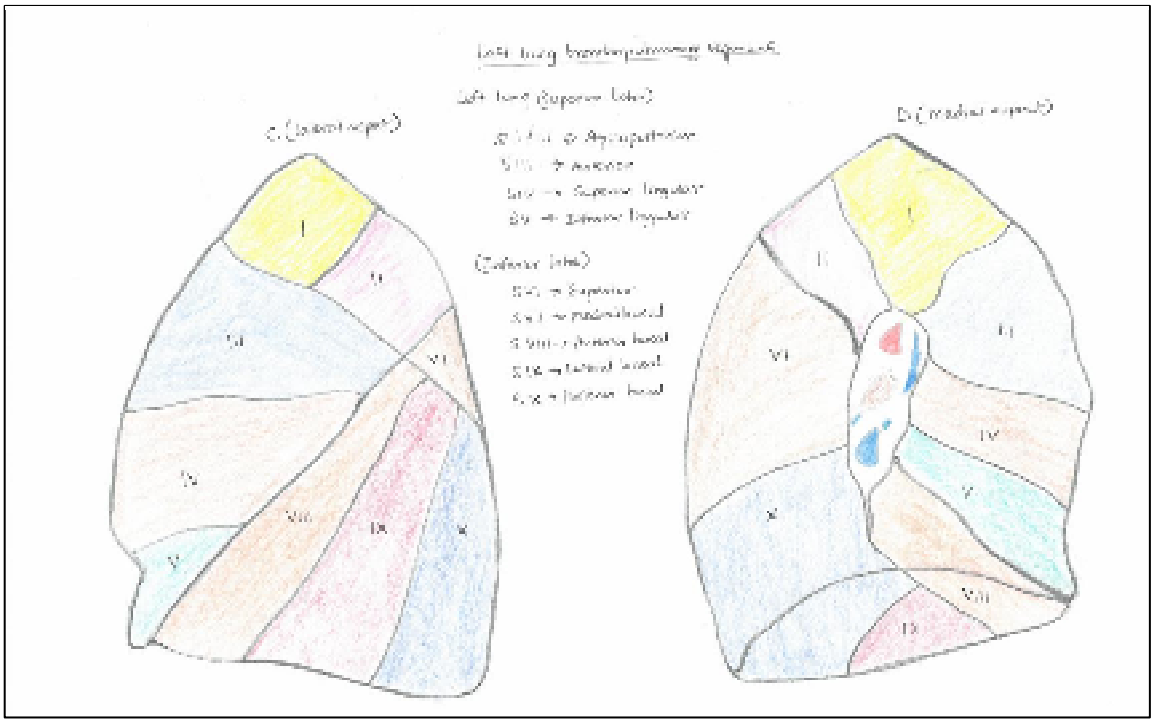


Fig.7.The left lung: Bronchopulmonary segments

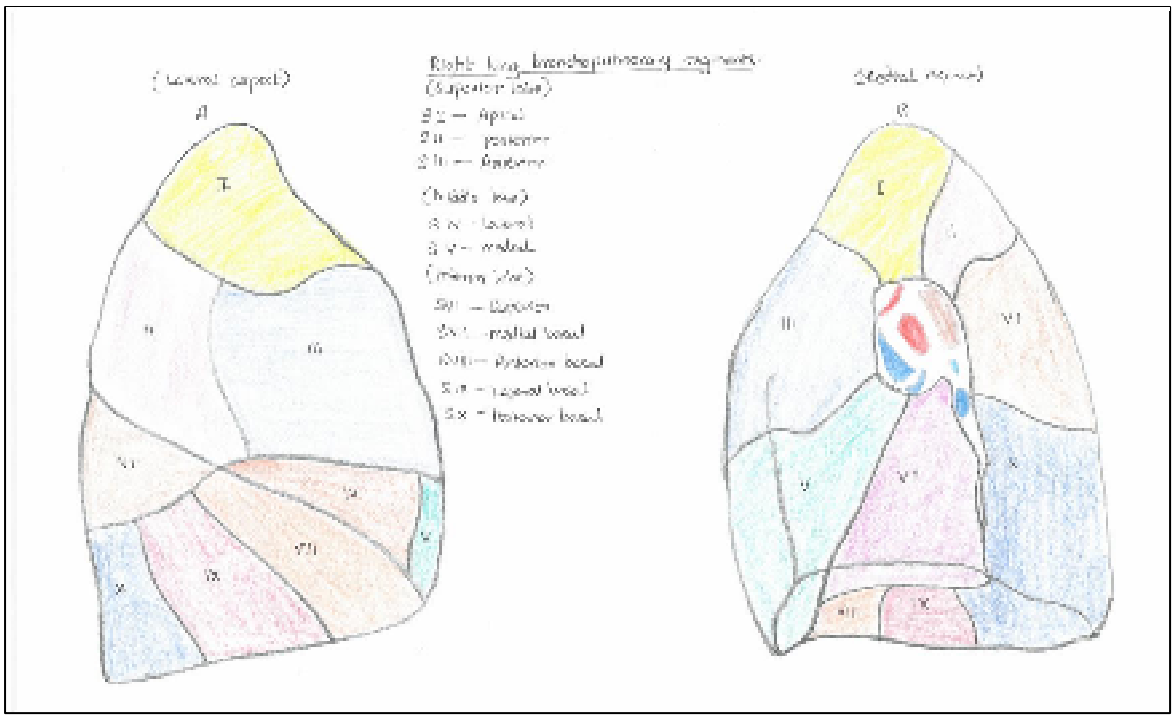


Fig.8.The right lung: Bronchopulmonary segments

Bronchi, Bronchioles and the lung lobule[8]

Bronchi are made up of cartilage and are greater than 1mm in diameter. Bronchioles are membranous and commonly less than 1mm in diameter. Proximal to the respiratory bronchioles, exist the non-respiratory bronchioles. Just in proximal location to the respiratory bronchioles, reside the terminal bronchioles that belong to the group of non-respiratory bronchioles. Respiratory bronchioles possess alveoli that are arranged by their walls in a budding fashion. The lung lobule is macroscopically evident structure and symbolizes the smallest subunit of lung that is enclosed by septae of connective tissue.

Vascular supply and Lymphatic drainage of lung[7-9]

The dual arterial blood supply is a chief feature of the lungs which consists of pulmonary arteries (and pulmonary veins) and bronchial arteries.

The pulmonary artery trunk carrying deoxygenated blood, arising from the right ventricle of heart gives two branches as right and left pulmonary arteries, which further enter lungs to form bronchovascular bundles.

The bronchial arteries originating from the descending part of thoracic aorta supply oxygenated blood to the lungs.

The pulmonary veins transport the oxygenated blood to the left atrial compartment.

The lymphatic channels originate in superficial and deep plexuses. There is absence of lymph vessels to alveoli. Superior lobes of lung and inferior lobes of lung have a tendency to drain into superior tracheobronchial nodes and inferior tracheobronchial nodes respectively.

The lymphatic channels of left lung ultimately drain into the thoracic duct. The right lung lymph fluid is finally drained into the right sided bronchomediastinal trunk. Lastly, both of them individually, drain into the left and the right subclavian veins respectively.

Normal Histology of Lung[8]

The surface epithelium rests on the basement membrane in large bronchi, beneath which there is an elastic layer of connective tissue. These components entirely constitute the bronchial mucosa.

The submucosa lies beneath the mucosa, where the structures like submucosal glands, nerves, cartilages and ganglia are found with the branches of bronchial artery.

A peribronchial sheath of loose connective tissue outlines the submucosa, that stays in continuity with the pulmonary artery accompanying it. The type of bronchial epithelium is pseudostratified columnar, chiefly comprising of ciliated columnar cells with scattered mucous cells.

Normally, few cells like neuroendocrine cells, brush cells, basal cells and migratory inflammatory cells are present in lesser numbers. The bronchiolar walls are thinner than the bronchial walls.

The basement membrane of airway has three layers which are the lamina lucida, lamina densa and the lamina reticularis. The cells of Basal, kulchitsky, ciliated, goblet, clara, intermediate and brush types are found to line the airways.

Goblet and ciliated cells go on decreasing quantitatively when they approach the terminal bronchioles. There is an accompanying rise in the clara cell population. The mucosa looks more cuboidal and less columnar. Clara cells possess surfactant like secretory function and after the injury to bronchioles, they come into play as

progenitors. Kulchitsky cells comprise of dense core granules and are a part of neuroendocrine cell family. Neuroepithelial cells tend to occur at airway bifurcation.

The functional unit of lung is the acinus, that plays a crucial role in the transfer of gases in and out of the body.

Residing by the walls of alveoli are the type I and type II epithelial cells, also termed as pneumocytes, which belong to squamous and cuboidal type of epithelium respectively. The cytoplasm of type I cells is associated with function of gaseous exchange. The type II cells are precursors of type I cells, that rejuvenate the integrity of alveolar epithelium in the post-injury phase. Surfactant production is one of the main functions of type II cells. The hyperplasia of type II pneumocytes is a non-specific highlighter of the wear and tear of alveoli. Percolating into the interstitium and scattered over the surfaces of alveoli are the macrophages. In the alveolar septum, there is a fusion of basement membrane of the epithelium and the capillary.

NON-SMALL CELL LUNG CANCERS -

NSCLCs are reported to be the commonest type of lung malignancies. Their occurence is frequent in 6^{th} to 7^{th} decade. Anatomic location of tumor and its size decides clinical symptoms. Early symptoms like cough, dyspnea and hemoptysis are inclined to be a part of centrally located tumors. For symptoms to occur, peripheral lung tumours need to be large in size. There is a linear correlation between the intensity of exposure to cigarette smoke and appearance of epithelial changes that begin with squamous metaplasia and progress to squamous dysplasia, carcinoma in-situ and invasive carcinoma. Most carcinomas arise by stepwise accumulation of genetic abnormalities that transform benign bronchial epithelium to neoplastic tissue.[1,2]

Histologic types of NSCLCs-

SQUAMOUS CELL CARCINOMA and Variants

Squamous cell carcinoma (SCC) is an epithelial tumour, malignant in nature, arising from bronchial epithelium exhibiting keratinization and / or intercellular bridge morphology.[1,10]

Over 90 % occur in cigarette smokers, usually males. The majority of squamous cell carcinomas, both keratinizing and non-keratinizing variant are central in location which are chiefly found inhabiting the mainstem, lobar or segmental bronchi.[1,2,10,11]

Macroscopy and Localization -

The gross appearance of these tumours is greyish to whitish in colour. Based upon the extent of fibrosis, they are firm to hard in consistency at the centre with stellate shaped retractions at the peripheral region. These tumours may show cavitations and a good chunk of these tumours tend to grow towards a larger size. The hallmark of these tumours is that they are polypoidal, intra-luminal masses that infiltrate the walls of bronchi including the circumferential tissues and obstruct the bronchial lumen, resulting in stasis of bronchial secretions, atelectasis, bronchial dilatation, obstructive lipoid pneumonia and infective bronchopneumonia.[10,11]

Histopathology -

Squamous cell carcinoma of keratinizing variant shows keratinization, pearl formation and intercellular bridges. These features vary with the degree of differentiation, being prominent in well-differentiated tumours and focal in poorly-differentiated tumours. Squamous cell carcinomas of non-keratinizing variant show diffuse positivity for p40 immunohistochemical stain which helps it in distinguishing from large cell carcinoma of null phenotype. Thus, keratinization being a hallmark of

squamous cell carcinoma, the differential diagnosis includes poorly differentiated non-small cell tumours or small biopsy specimens with limited tumour tissue that show no morphological features of squamous cell differentiation.[1,10]

Papillary SCC - In some of the proximally located tumours, features such as exophytic and endobronchial growth may be seen. They are delicate intrapapillary lesions with little or no stromal invasion and no necrosis.[2,11]

Clear cell SCC - This variant shows cells that have clear cytoplasm but exhibit evidence of keratinization. The differential diagnoses for this variant are large cell carcinoma, adenocarcinoma of the lung with extensive clear cell change and metastatic renal clear cell carcinoma.[2,11]

Small cell SCC - They are poorly differentiated, show small tumour cells with morphologic characteristics of a non-small cell carcinoma along with focal squamous differentiation. They lack the characteristic nuclear features of small cell carcinoma having coarse or vesicular chromatin, more prominent nucleoli, more cytoplasm and more distinct cell borders. Focal intercellular bridges or keratinization can be seen.[2,11]

Basaloid variant - A salient microscopic feature is the peripheral palisading of nuclei. Poor differentiation and extensive basaloid pattern that lacks squamous differentiation are the characteristic attributes of basaloid variant.[2,10,11]

ADENOCARCINOMA and Variants[1,2,10]

Invasive adenocarcinoma is a malignant epithelial tumour with glandular differentiation, mucin production, or pneumocyte marker expression.

Macroscopy and Localization -

The most common localization is in the lung periphery. They appear as grey white nodules with central scarring fibrosis which is associated with anthracotic pigmentation and pleural puckering.

Histopathology -

Invasive adenocarcinomas consist of complex heterogenous mixture of histological subtypes. So the term pre-dominant is applied to all categories of invasive non-mucinous adenocarcinomas which further replaces the term adenocarcinoma-mixed subtype.

Lepidic adenocarcinoma - consists of bland pneumocytic cells (type II pneumocytes or clara cells) that grow along the surface of alveolar wall.

Acinar adenocarcinoma - shows round to oval shaped malignant glands invading a fibrous stroma arranged in a cribriform pattern.

Papillary adenocarcinoma - shows a major growth of malignant cuboidal to columnar glandular cells in papillary fashion along central fibrovascular cores.

Micropapillary adenocarcinoma - shows the tumour cells growing in papillary tufts forming florets that lack fibrovascular cores.

Solid adenocarcinoma - shows polygonal tumour cells forming sheets that lack recognizable patterns of adenocarcinoma as the acinar, papillary, micropapillary or lepidic growth.

Invasive mucinous adenocarcinoma - shows cells that demonstrate goblet or columnar type of morphology and ample intracytoplasmic mucin.

Macroscopy and localization - They occur in lung periphery. They appear poorly circumscribed, soft and gelatinous tumour without central desmoplastic fibrosis and anthracotic pigmentation.

Histopathology - The tumour formerly called as mucinous bronchioalveolar carcinoma, shows tumour cells that possess goblet and/or columnar type of morphology with ample intracytoplasmic mucin and small oriented nuclei basally.

Colloid adenocarcinoma - shows replacement of air spaces by copious mucin pools.

Macroscopy and localization - They occur mostly as peripheral masses. A soft and gelatinous well demarcated nodule with uni- to multi- locular formation is seen.

Histopathology - They show copius mucin pools extracellularly that tend to distend alveolar spaces, further destroying their walls by displaying invasive pattern of growth into the adjacent alveolar spaces. Presence of scant bland tumour cells singly or in clusters floating within the mucin pools may be seen.

Fetal adenocarcinoma - it is an adenocarcinoma that resembles a fetal lung.

Macroscopy and localization- Usually peripheral in location.These lesions are solitary, well demarcated pulmonary mass lesions. The cut surface is bulging and appears white to tan-brown with areas of cystic change and hemorrhage.

Histopathology - The tissue shows presence of glandular structures, which comprises of plenty of glycogen, non ciliated cells that resemble an epithelium of pseudoglandular phase of fetal lung.

Enteric adenocarcinoma - It resembles the adenocarcinoma arising in the colorectum.

Minimally invasive adenocarcinoma -

Macroscopy and localization - Occur usually in the lung periphery. They show central collapsed fibrosis and tumour with visible alveolar air spaces.

Histopathology-

It is a small < 3cm or =3 cm solitary adenocarcinoma with predominat lepidic pattern, <5mm or =5mm invasion in greatest dimension.

It should be excluded if tumour invades lymphatics, blood vessels, air spaces or the pleura.

NEUROENDOCRINE TUMOURS[1,2,10]

Small cell lung carcinoma -

Macroscopy and localization - They present as a large perihilar mass with peribronchial compression and nodal involvement.

Histopathology-The tumour cells are round to oval or spindle shaped, are tightly packed and are small with scanty cytoplasm, fine granular chromatin with absent nucleoli. Mitoses can be seen at foci.

Large cell neuroendocrine lung carcinoma -

Macroscopy and localization - Large circumscribed masses that frequently occur in periphery and upper lobes. Cut surface shows necrotic tan-red surface.

Histopathology - They show nesting, trabecular growth, rosette-like structures and peripheral palisading pattern with large areas of necrosis. Presence of brisk mitotic activity is noted.

CARCINOID TUMOURS[1,2,10]

Macroscopy and localization-They can be found from trachea to bronchioles. They are well circumscribed, round to ovoid in shape and sessile.

Histopathology-

Typical carcinoids are tumours with <2 mitoses per 2 mm^2 with no necrosis.

Atypical carcinoids are tumours with 2-10 mitoses per $2mm^2$ with few necrotic foci.

LARGE CELL CARCINOMA[1,2,10]

Macroscopy and localization - Usually large, solid and circumscribed masses seen often with necrosis and located peripherally.

Histopathology -They comprise of sheets and nests of large polygonal cells with vesicular nuclei, prominent nucleoli and moderate amounts of cytoplasm.

ADENOSQUAMOUS CARCINOMA[1,2,10]

Macroscopy and localization-They occur peripherally and gross features are similar to NSCLCs.

Histopathology-They show combination of morphology of squamous cell carcinoma and adenocarcinoma, with each component constituting atleast ten percent of the tumour.

CARCINOSARCOMA[1,2,10]

Macroscopy and localization-Usually centrally located and present as grey-white, necrotic and hemorrhagic masses.

Histopathology-They are the tumours with intimately admixed non-small cell carcinoma and sarcoma containing heterologous elements.

SPINDLE CELL CARCINOMA[10]

It comprises of epithelial spindle cells with no differentiated carcinomatous elements. They show malignant spindle shaped cells in fascicular or storiform patterns with hyperchromatic nuclei with nucleoli and granular type of chromatin.

EGFR IN LUNG CANCERS

Discovery of EGFR [12]

EGF-specific receptor was first reported to be located on the cell membrane of the fibroblast in 1975. Later, by research on A431 human cancer cell line, the EGFR was defined as a 170 kDa protein.

In the year 1984, the v-erbB, an oncogene present in the avian erythroblastic leukemia virus, was found to have near similarity to EGFR.

Later, it was reported that genes related to v-erbB were not only EGFR but also were human epidermal growth factor receptor 2(HER2), labelled as ERBB1 and ERBB2 respectively.

Structure and functions of EGFR [12-18]

Growth factors are polypeptides that impact proliferation and differentiation in the normal and malignant cells.

EGF was one of the first growth factors to be discovered. Binding of ligand to EGFR stimulates conformational changes in the receptor that progressively increases the catalytic action of the intrinsic tyrosine kinase which results in the auto-phosphorylation essential for biologic activity.

The erbB family cell signaling process utilizes EGF-like ligands that possess cell signaling transforming growth factor α (TGF α), amphiregulin, heparin binding EGF, epiregulin, heregulin, neuregulin and betacellulin. EGFR is best-known to have high affinity binding to EGF, amphiregulin and TGF α.

EGFR belongs to the erbB family of receptor tyrosine kinase proteins, that include HER2/neu (erbB2), HER3 (erbB3) and HER4 (erbB4). These receptors possess extracellular ligand-binding domain, a transmembrane lipophilic domain and

an intracellular tyrosine kinase domain that all bind to receptor specific ligands with the exception of HER2.

The phosphorylation of the tyrosine kinase domain followed by homodimerisation or heterodimerisation between the receptors of the same family causes protein activation on the cell surface. This is considered to hike the signaling cascades, cell growth, differentiation, cell survival, cell cycle progression and angiogenesis mechanisms in the cancer cells.

Genetic structure of EGFR [12-18]

The two hundred kb human EGFR gene comprises of 28 exons and 27 introns and is present on the short arm of chromosome 7(7p12). Exons 1-16 code for the extracellular domain, whereas exon 17 codes for the transmembrane domain. Exons 18-28 are accountable for the intracellular domains. Exons 18-24 code for the tyrosine kinase domain. The domain labelled as C-terminal is encoded by exons 25-28.

Activation of EGFR downstream signaling [12-18]

The EGFR, which belongs to receptor tyrosine kinases, transfers extracellular signals of growth factors into the intracytoplasmic regions and conveys its stimulus to the nuclei by signal transduction. Further, a transcriptional upregulation occurs that leads to the synthesis of protein and alteration the of cell functions and/or architecture.

The Ras/Raf/MAPK (Mitogen-Activated Protein Kinase)pathway, the PI3K(Phosphatidylinositol-3-Kinase) /Akt pathway and the Jak (Janus kinase)/ STAT(Signal Transducers And Activator of Transcription) pathways are crucial in the EGFR signaling. Further, the cell proliferation and/or cell differentiation are boosted. There is a promotion of cell proliferation and survival by Ras/Raf/MAPK pathway, whereas the PI3K/Akt pathway is principally related to cell growth, inhibition of apoptosis and invasion or migration.

EGFR Overexpression[12]

Till date, EGFR overexpression has been noticed in various cancers within a wide scope of solid cancers. Reported values are 30%-38% for adenocarcinomas of stomach, 30%-60% for pancreatic cancers and 100% for thyroid carcinomas of undifferentiated type.

Extracellular mutation of EGFR[13,15,16]

It was found in 1988 that human glioblastoma multiforme cells carried amplified c-erbB genes which consisted of short deletion mutations inside the ligand-binding domain of the EGFR. The mutated c-erbB gene products were reported to be 30-kDa smaller than 170-kDa.

The EGFR and the cancer cell membrane parts comprising of the 140-kDa abnormal EGFR displayed an ascent of tyrosine kinase activity without any ligand. This type of mutation was labelled as EGFR vIII. EGFR vIII is found to be associated with cell proliferation and malignancies involving cancers of breast, small cell lung cancers, gliomas and prostatic cancers.

Mutations of intracytoplamic domain of EGFR gene[12,13,15-18]

Mutations of intracytoplasmic domain of EGFR gene were found in NSCLCs in the year 2004. NSCLCs with these mutations were ablated in size by gefitinib, a chemical inhibitor of the tyrosine kinase. Considering the gene coding for the receptor, the mutations are divided into four major types:

-point mutations in exon 18

-deletions in exon 19

-insertions in exon 20

-point mutations in exon 21

The two predominant mutations were deletion around codons 746-750 of exon 19 and transversion of T to G in codon 858 of exon 21, with an amino acid change from leucine to arginine (L858R). These two mutations accounted for about 90% of intracytoplasmic mutations of EGFR. Both of these mutations were found to cause conformational change in the ATP-binding domain, which results in invariable activation of EGFR without the ligand binding. These two EGFR mutations have been found to be present in normal lung tissue around cancers. Mice that are transgenic for the mutated EGFR gene, acquire lung cancers. This finally declares that EGFR mutation is present from an early stage of neoplasia in the lung.

Drugs targeting EGFR [12-16,19-26]

The innovative EGFR inhibition strategies include small-molecule inhibition of the intracellular tyrosine kinase domain and monoclonal antibody-mediated blockade of the extracellular ligand-binding domain. Gefitinib and erlotinib are the oral anticancer drugs that inhibit tyrosine kinase domain. They possess cytoreductive effects that may be dependant on intracytoplasmic mutations of EGFR. They have been found to be highly effective in treating the NSCLCs.

In many studies done retrospectively, the patients with EGFR mutations responding to gefitinib and/or erlotinib showed a longer progression-free survival which helped in improved overall survival as opposed to the patients whose tumours had wild-type EGFR.

Studies in recent years found that EGFR mutations are related to significant survival in the patients with resectable lung adenocarcinomas those who are not treated with EGFR TKI's. Thus, EGFR mutations possess a significant predictive and

prognostic value. Evidence of activating EGFR mutations is the best predictor for EGFR-TKI response.

Clinical heterogeneity of EGFR mutations [13,27,28]

Few mutations had favourable responses to EGFR TKIs, whereas few were associated with poor responses and/or resistance to the same drugs. Hence, the comparatively exceptional in-frame insertions within exon 20 have a primary resistance to the EGFR TKIs.

Two retrospective comparative studies showed the outcome of EGFR TKI-treated patients based on the type of mutations that chiefly included exon 19 deletions and/or exon 21 L858R point mutations. It was found that the first group type had a better overall survival.

IHC -EGFR and targeted therapy

Both adenocarcinomas and squamous cell carcinomas are surgically intervened if resectable. Addition of the adjuvant radiotherapy and chemotherapy in the treatment protocol if necessary, is to be done. Clinical stage of the disease is the most crucial determinant of the prognosis of lung cancer.[29]

Immunohistochemistry plays a chief role in the classification of tumours into subtypes and to measure biomarkers to simplify therapeautic decisions. When compared to other methods, IHC has many advantages such as wide range availability, less technical challenges, cost efficiency and rapid turn-around time. Immunohistochemistry can be interpreted using fewer tumour cells as compared to the molecular methods. Also, the immunohistochemistry helps in evaluating staining patterns and localisation of specific cells in the analysable tumour structures. IHC has a huge scope to be used as a screening tool for spotting specific genetic alterations related to molecular based targeted therapies.[4,30-36]

One of the commonest driver mutations in adenocarcinoma of lung is the EGFR mutation. Increased EGFR gene copy number by FISH and EGFR protein overexpression measured by IHC are found to correlate with improved response and survival with gefitinib and cetuximab treatment. Based on the IHC and molecular tumour characterization, the management of non-curable NSCLCs is becoming specific and more individualised, thus EGFR testing plays a major role in NSCLC treatment.[37-42]

Six randomized control trials have demonstrated a higher tumour response rate and a longer progression free survival in EGFR-mutant subjects on treatment with the first line TKIs.[43-49]

Most of them developed gefitinib or erlotinib failure. On treatment failure, many patients had secondary EGFR T790M mutation, c-MET amplification or both. Second somatic activating mutations, especially T790M was responsible for around 50% of the aquired resistance post-TKI in EGFR mutated lung cancers.[50-58]

One of the recent study showed that prevalence of family history of lung cancer in first degree relatives was 9%.[59]

Scoring systems for EGFR expression-

Current scoring methods, use internationally validated antibodies that help in detection of the EGFR expression levels in the tumour samples. Few studies showed that the EGFR expression levels that were determined by IHC, were tested as a biomarker to assess the efficacy of cisplatin and vinorelbine plus cetuximab.[60-63]

EGFR expression by FLEX study (The updated H-score method)-

The tumour sample scoring was done according to the different cell staining intensities. The score ranged from 0-3. The 4 categories were 0(no staining), 1(weak), 2(intermediate), 3(strong). Strong stain was easily visible under 4x objective,

moderate stain was visible by 10x or 20x objective with lucid visibility and weak stain visibility needed 40x objective. H-score was defined as continuous variable with range of 0-300 and was calculated using the formula= (1x %of weakly stained cells)+ (2x %of intermediate stained cells) + (3x %of strongly stained cells). The threshold was set as H-score of 200.[64] H-score of >=200 was considered IHC positive and <200 score was taken as IHC negative.

This method of FLEX study by Pirker et al[64] uses EGFR IHC evaluation by H-score with magnification rule. This rule includes intensities visualized at different magnifications.[65]

EGFR expression method by Hirsch et al-[66]

This method used one magnification (10x) system for evaluating staining intensities of cell membranes and scoring. Only clear staining of the tumour cell membranes was considered positive. A semiquantitative approach was used to generate an overall score ranging from 0-400. The percentage of positive tumour cells per slide (0%-100%), was multiplied by dominant intensity pattern of staining: 1-negative, 2-weak, 3-moderate, 4-intense. Specimens with low levels of expression were revaluated for detecting analysable tumour cells under 40 x objectives.

Method of scoring EGFR IHC by Atkins D[67]

The staining pattern of tumour cell membranes was considered as incomplete when only part of their membrane was stained and complete when cells showed circumferential staining of the entire cell membrane. The scoring approach was: score 0- no staining or unspecific, 1- weak intensity and incomplete staining(quality) of more than 10% of tumour cells (quantity), 2-moderate and complete staining of more than 10% of tumour cells, 3- strong and complete staining of more than 10% of tumour cells.

SATURN protocol scoring method for EGFR IHC

This method classifies samples into two main groups as IHC positive and IHC negative. If more than 10% of tumour cells show membranous staining of any intensity, it is considered as IHC positive, whereas less than 10%, it is negative.[65]

METHODOLOGY

The present study has been carried out at the Pathology Department of KAHER's Jawaharlal Nehru Medical College and Dr. Prabhakar Kore Hospital and Research Centre, Belagavi.

Study design: Descriptive observational study

Study Period: January 2019-December 2020

Study Population: All cases of surgically resected lung tumour specimens and biopsies from the patients who have undergone operative procedures at KLES DR.PRABHAKAR KORE HOSPITAL.

For retrospective cases, data as well as tissue blocks were retrieved from storage section.

Inclusion criteria: Well fixed surgically resected specimens and biopsy specimens diagnosed as carcinoma lung.

Exclusion criteria:

1.Inadequate biopsies.

2. Improperly fixed specimens.

3.Recurrent/Previously operated cases.

Sample Size Calculation: The sample size was calculated using the formula

$N= 4pq/d^2$, p= Expression of EGFR, q=100-p, d= Sample error (10).

Substituting the values in the above formula a sample size of 40 was obtained.

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human subjects Research. (Ref.:MDC/DOME) (Annexure III)

Sample Size: 40

Data Collection and Case selection

The present study was conducted on Lung carcinoma cases admitted in KLES Dr. Prabhakar Kore Hospital & Medical Research Centre, Belagavi. After obtaining permission from the hospital authorities, the participants will be briefed about the study. Informed consent will be obtained from all the prospective (new) cases. Information regarding old cases will be obtained from Medical Records Department (MRD). The data will be collected using a predesigned, pretested proforma. The information regarding clinical parameters including age, history, surgery type will be obtained from the patient's records from Medical Records Department(MRD)as per proforma given in Annexure II and that of the gross appearance will be obtained from the grossing notes from the Department of Pathology, KAHER. The specimens were adequately fixed in 10% neutral buffered formalin. 4-5 micron thick sections were cut from paraffin embedded blocks. One section from each block was taken for staining with Haematoxylin & Eosin (H&E) followed by Immunohistochemistry for Epidermal Growth Factor Receptor (EGFR) as per standardized procedures given in Annexures IV and V. Its expression will be evaluated semiquantitatively by assessing the intensity and by calculating the H-score under magnifications 4x, 10x and 40x objectives.

The tumours were typed according to the WHO 2015 classification system.

All forty cases were then studied for expression of EGFR by IHC.

Digital images were obtained.

Assessment of expression of EGFR[64]

The expression of EGFR was assessed semiquantitatively by evaluating intensity at a score of 0-3 & estimation of percentage of positive tumour cells showing membrane staining at all intensities.

Table 1: Scores for EGFR staining intensity

0	"No staining"
1+	"Weak", light-brown membrane staining and visible only under high magnification
2+	Intermediate staining between 1+ and 3+
3+	"Strong", dark-brown linear membrane staining and visible under low magnification

Thus, a score of >= 1 was considered positive, and a score of 0 was negative.

H-score with a range of 0-300 was used and was calculated using the formula= (1x %of weakly stained cells+ 2x %of intermediate stained cells+ 3x %of strongly stained cells). The threshold was set as H-score of 200.

H-score >=200 was considered IHC positive and <200 considered negative.

Statistical Analysis:

Data will be analysed using SPSS software version-26. The data concluded will be expressed in percentages. Descriptive analysis has been performed in the present study and the analysis of data was done using the statistical software stata 14.2. Microsoft word and Excel have been employed to generate graphs, tables etc. Descriptive statistics such as mean & percentages were calculated. The relationship between expression of EGFR marker and other clinico-pathological variables was analysed using chi-square test.

Probability (P) value: < 0.05 was considered statistically significant.

RESULTS

A descriptive observational study was carried out in the Pathology Department of KAHER's Jawaharlal Nehru Medical College and Dr.Prabhakar Kore Hospital and Research Centre, Belagavi to study the IHC expression EGFR in lung cancer cases.

A total of 40 cases were evaluated. The data obtained from the study was compiled, tabulated and subjected to statistical analysis. The results are presented here under the headings of the various parameters considered for the study.

Table 2: Demographic profile of patients

Demographic profile	No of patients	% of patients
Age groups		
<=50yrs	7	17.50
51-60yrs	16	40.00
61-70yrs	11	27.50
>=71yrs	6	15.00
Mean Age(yrs)±SD	58.90±13.95	
Gender		
Male	27	67.50
Female	13	32.50
Total	40	100.00

Peak incidence was reported in 51-60 yrs with 16(40%) patients in total, followed by 11(27.50%) patients in the age group of 61-70 years.
The mean age calculated was 58.90 yrs(±13.95 SD).
The median age calculated was 60 years.

Out of the 40 cases, 27(67.50%) were males and 13(32.50%) were females.
Male:female (M:F) ratio was 2.08:1.

Graph 1: Demographic profile of patients

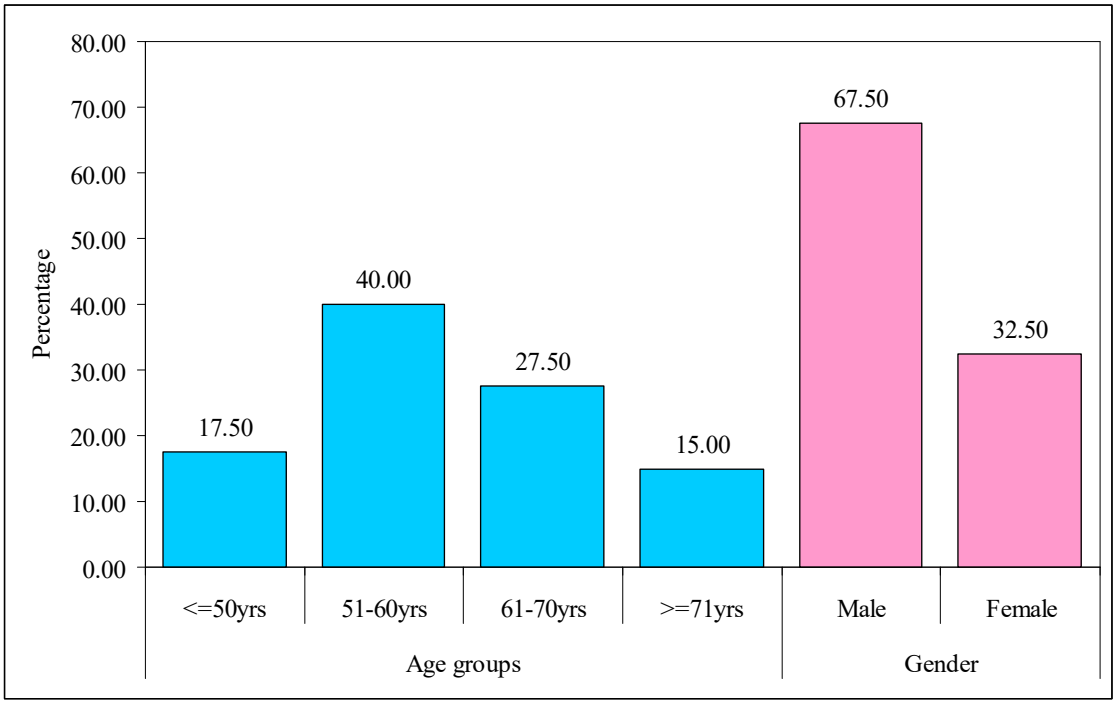


Table 3: Site of tumour wise distribution of cases

Site of tumour	No of patients	% of patients
Right upper lobe	20	50.00
Right lower lobe	9	22.50
Left upper lobe	5	12.50
Left lower lobe	6	15.00
Total	40	100.00

Majority of the patients had tumour located in the upper lobe of right lung [20 cases (50%)] followed by lower lobe of the right lung [9 cases (22.50%)].

Chart 1: Site of tumour wise distribution of cases

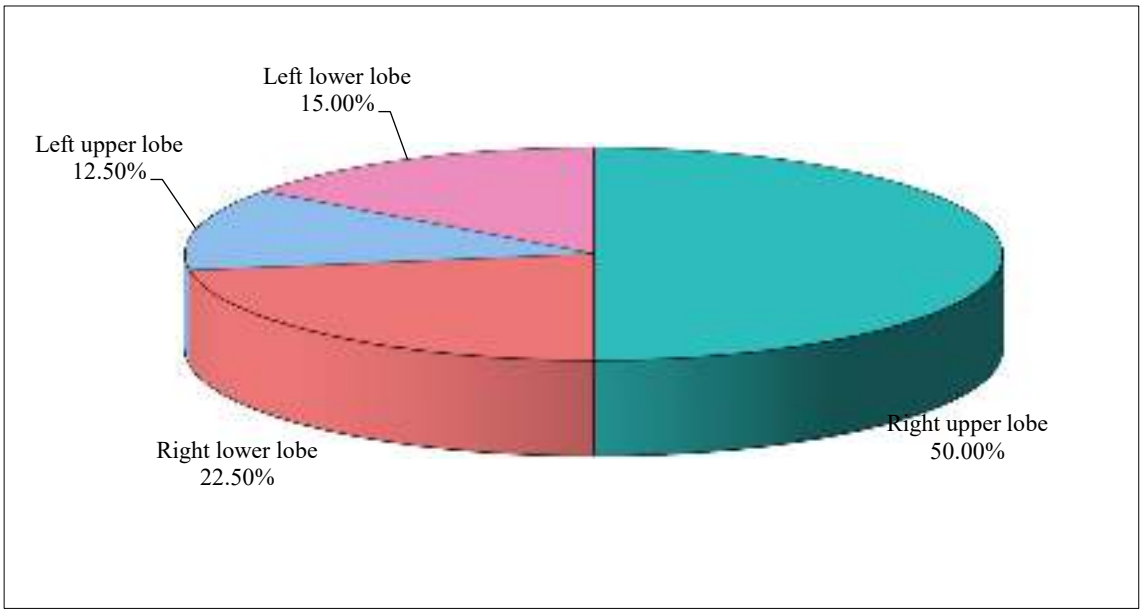
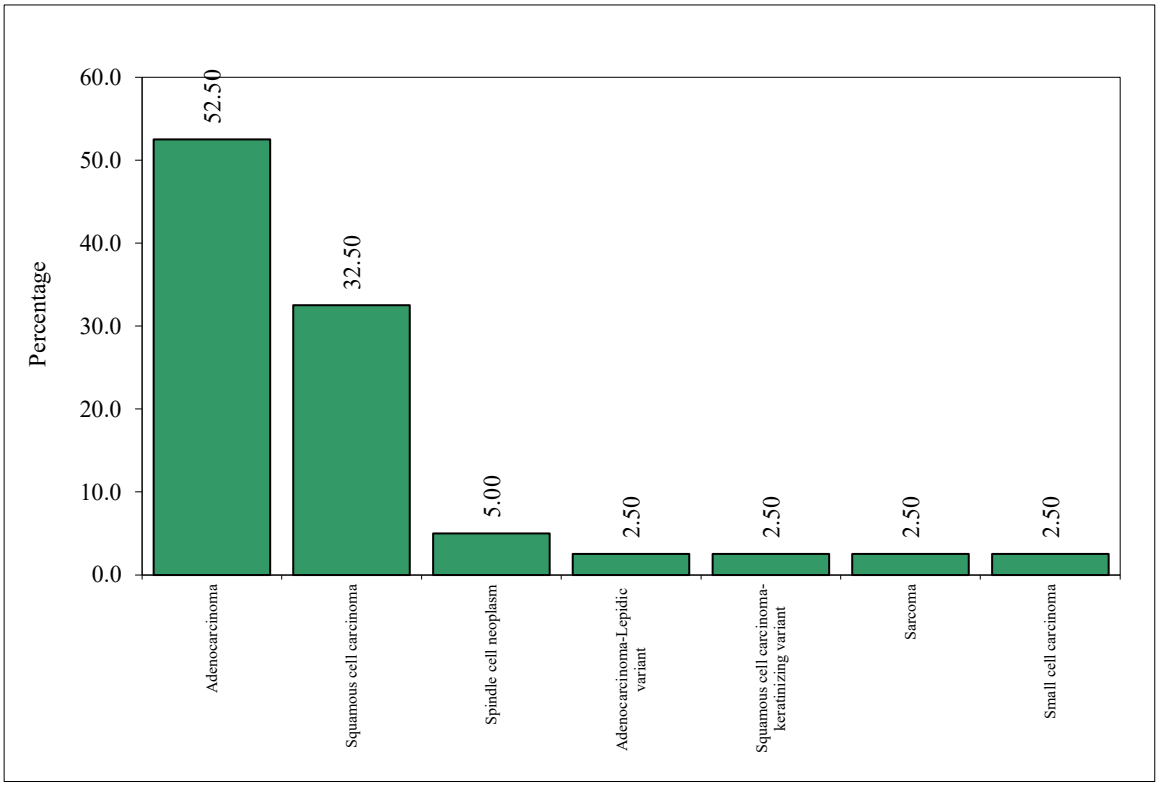


Table 4: Distribution of cases based on types of tumour

Type of carcinoma	No of patients	% of patients
Adenocarcinoma	21	52.50
Squamous cell carcinoma	13	32.50
Adenocarcinoma-Lepidic variant	1	2.50
Squamous cell carcinoma-keratinizing variant	1	2.50
Sarcoma	1	2.50
Small cell carcinoma	1	2.50
Spindle cell neoplasm	2	5.00
Total	40	100.00

Graph 2: Distribution of cases based on types of tumour



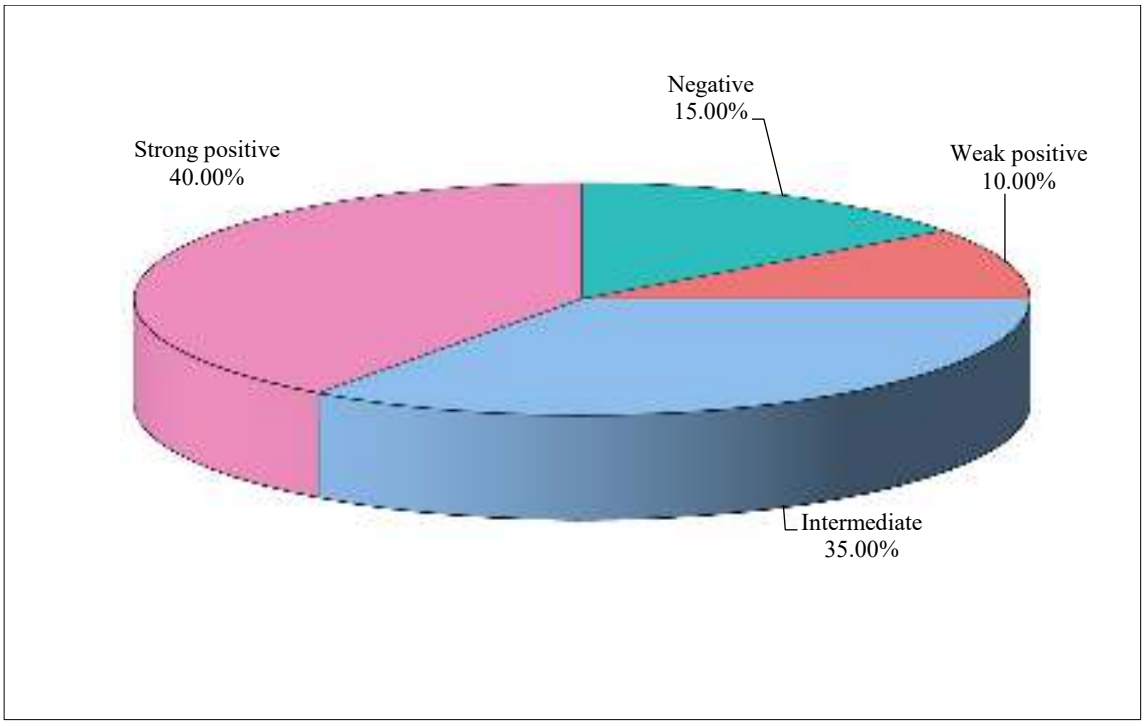
Adenocarcinoma accounted for maximum number of cases = 21(52.50%).

Table 5: EGFR staining intensity wise distribution of cases

EGFR staining intensity	No of patients	% of patients
Negative	6	15.00
Weak positive	4	10.00
Intermediate	14	35.00
Strong positive	16	40.00
Total	40	100.00

Majority of 16(40%) cases showed strong positive EGFR staining. 6(15%)cases showed negative EGFR staining.

Chart 2: EGFR staining intensity wise distribution of cases



H-score (Histo-score) was defined as continuous variable with range of 0-300 and was calculated using the formula = [1 x (%of weakly stained cells)] + [2 x (%of intermediate stained cells)] + [3x (%of strongly stained cells)]. The threshold was set to H-score of 200.

Table 6: H-score wise distribution of cases

H-score	No of patients	% of patients
>=200	29	72.50
<200	11	27.50
Total	40	100.00

29/40 (72.50%) cases had H-score of >=200.

Chart 3: H-score wise distribution of cases

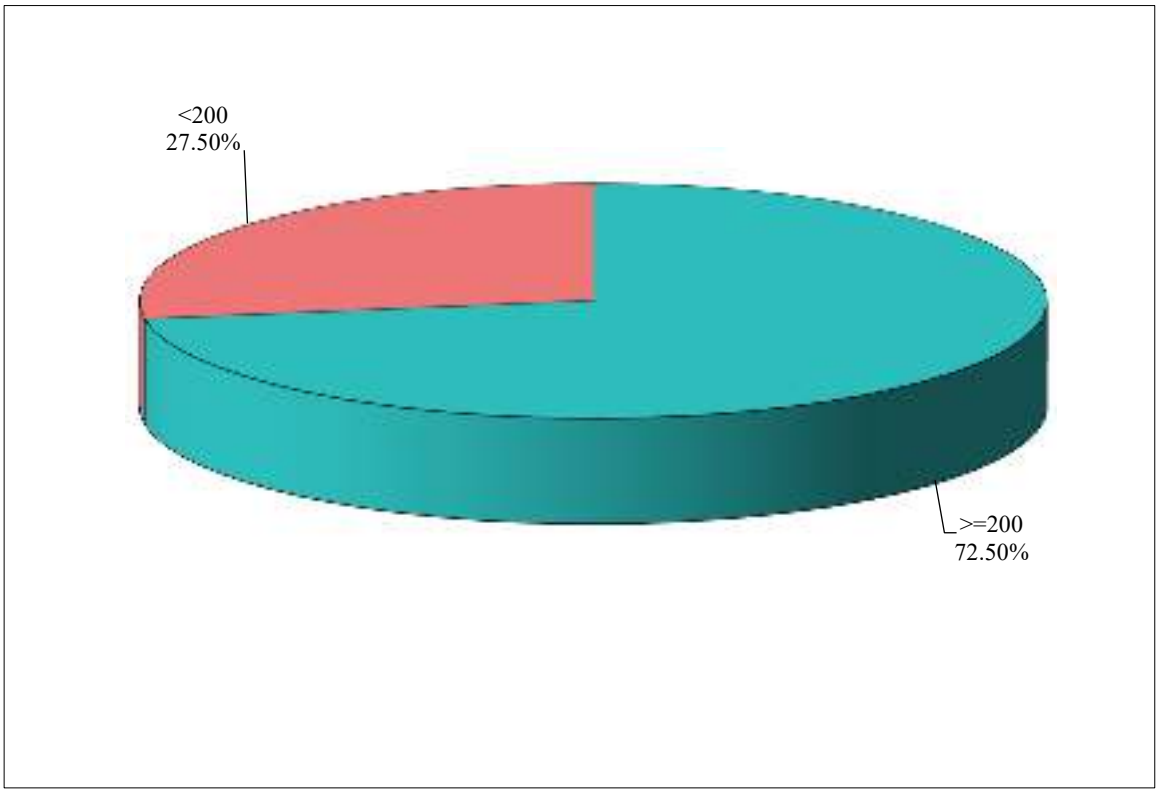


Table 7: Smoking status, Diet and Family history wise distribution of cases

	No of patients	% of patients
Smoking status		
Yes	26	65.00
No	14	35.00
Diet		
Vegetarian	19	47.50
Mixed	21	52.50
Family history		
Present	6	15.00
Absent	34	85.00
Total	40	100.00

Out of 40 cases, 26(65%) had a positive smoking status, 21(52.50%) patients had mixed diet intake and 6(15%) had positive family history for lung cancer.

Graph 3: Smoking status, Diet and Family history wise distribution of cases

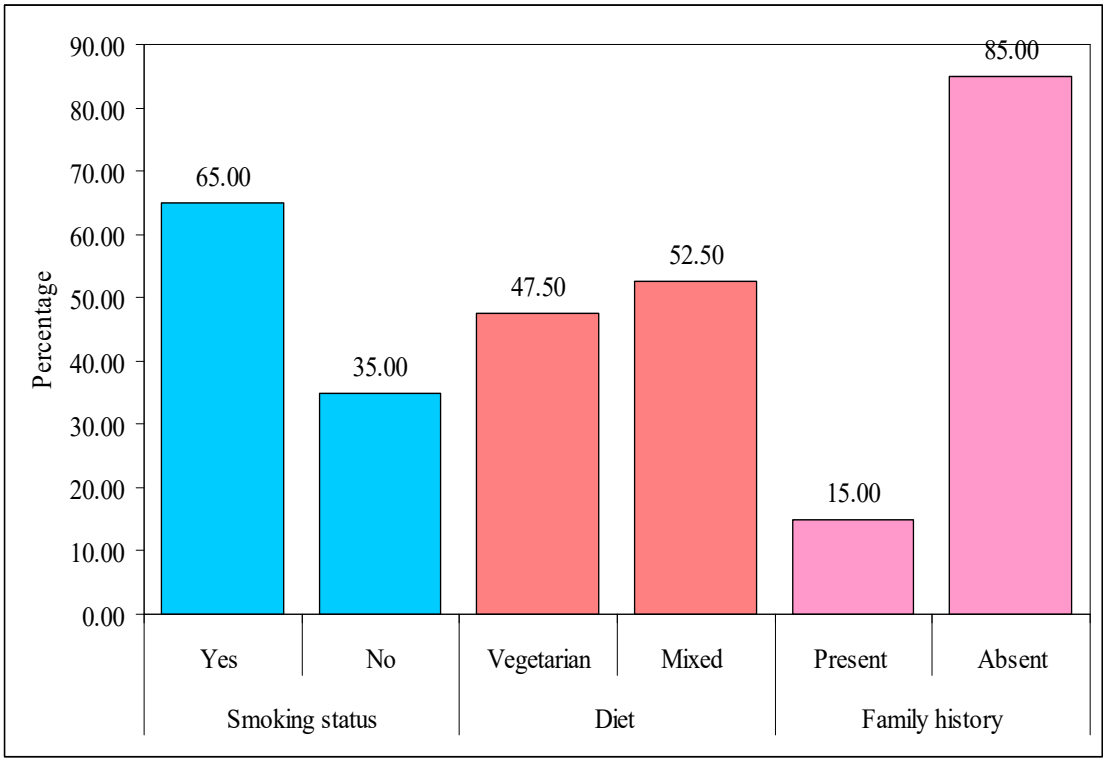


Table 8: Symptoms wise distribution of cases

Symptoms	No of patients	% of patients
Cough		
Present	35	87.50
Absent	5	12.50
Dyspnoea		
Present	25	62.50
Absent	15	37.50
Loss of appetite		
Present	25	62.50
Absent	15	37.50
Weight loss		
Present	27	67.50
Absent	13	32.50
Hemoptysis		
Present	18	45.00
Absent	22	55.00
Total	40	100.00

Out of the total 40 cases, 35(87.50%) patients had cough as a major symptom. Dyspnoea and loss of appetite were reported in 25(62.50%) patients each. Weight loss was noted by 27(67.50%) patients. Hemoptysis was present in 18(45%) cases.

Graph 4: Symptoms wise distribution of cases

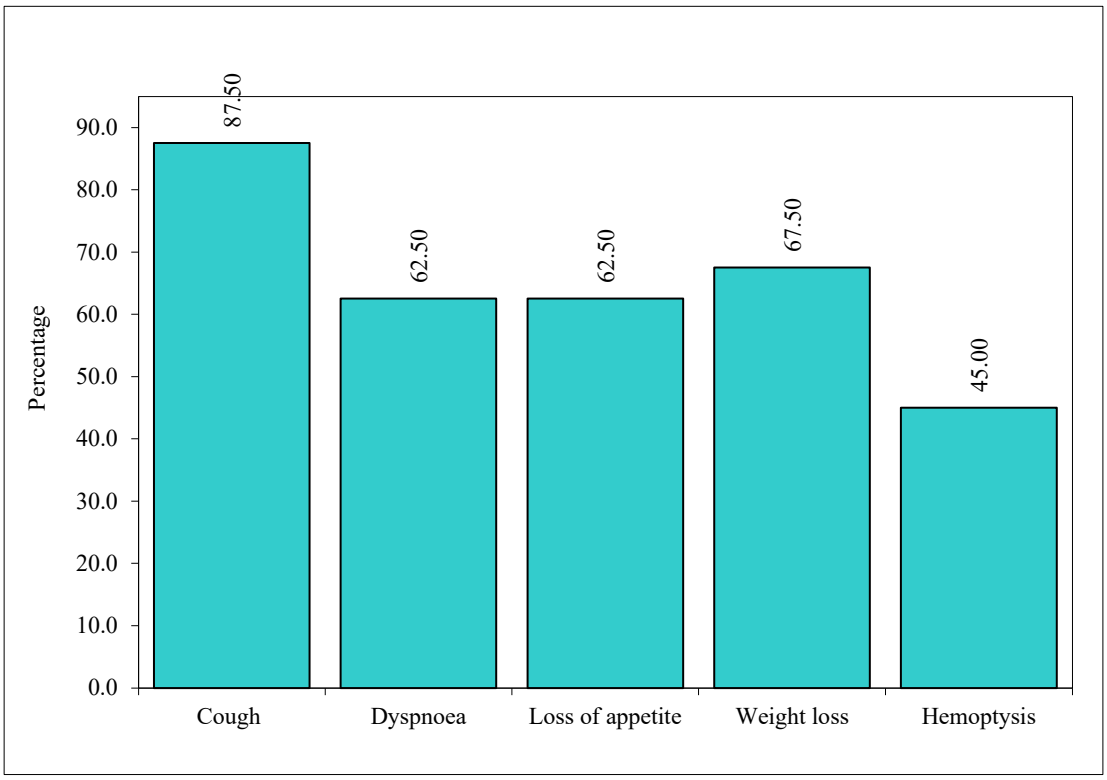
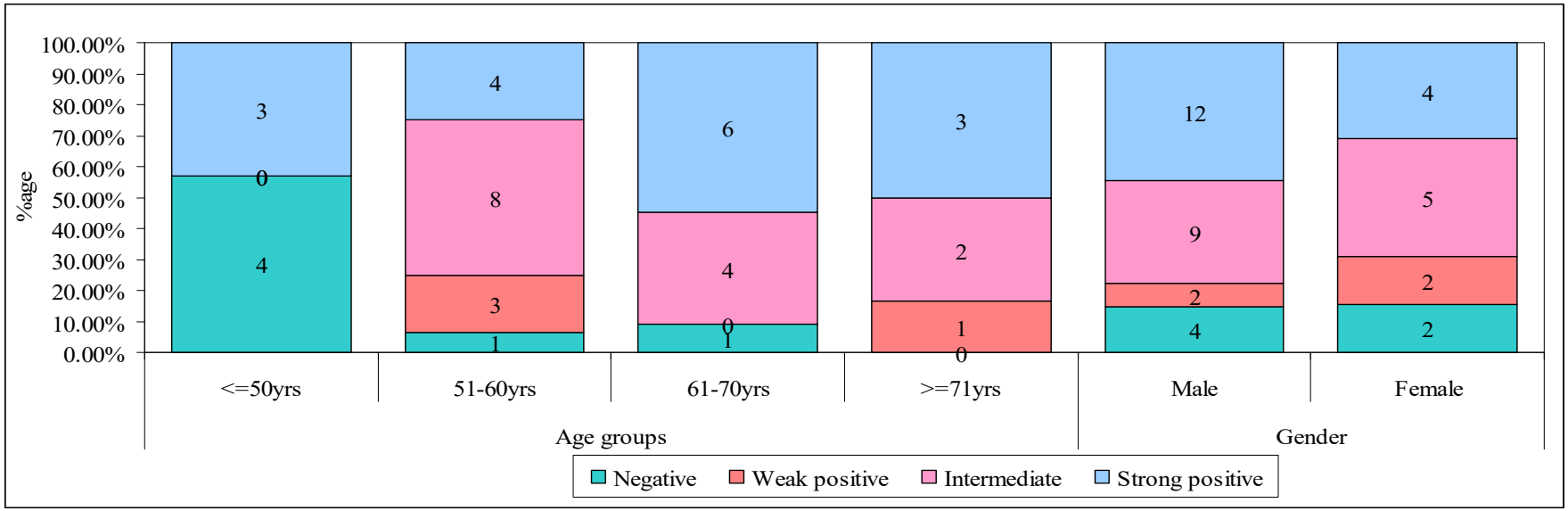


Table 9: Association of demographic profile with EGFR Staining intensity

	Negative	%	Weak +ve	%	Intermediate	%	Strong +ve	%	Total	%	χ^2	p-value
Age groups												
<=50yrs	4	57.14	0	0.00	0	0.00	3	42.86	7	17.50	18.6880	0.0280*
51-60yrs	1	6.25	3	18.75	8	50.00	4	25.00	16	40.00		
61-70yrs	1	9.09	0	0.00	4	36.36	6	54.55	11	27.50		
>=71yrs	0	0.00	1	16.67	2	33.33	3	50.00	6	15.00		
Gender												
Male	4	14.81	2	7.41	9	33.33	12	44.44	27	67.50	1.0360	0.7920
Female	2	15.38	2	15.38	5	38.46	4	30.77	13	32.50		
Total	6	15.00	4	10.00	14	35.00	16	40.00	40	100.0		

*p<0.05

Graph 5: Association of demographic profile with EGFR Staining intensity



Out of the total cases distributed in various age groups, majority of 4(57.14%) in <=50yrs group, 8(50%) in 51-60 yrs group, 6(54.55%) in 61-70 yrs group and 3(50%) in >=71 yrs group showed EGFR staining intensity of negative, intermediate and strong positive for the latter two age groups respectively. The 'p' value was 0.0280 ($p<0.05$). The result was statistically significant.

Out of the total cases distributed in the gender groups, majority of 12(44.44%) in male group and 5(38.46%) in female group showed EGFR staining intensity of strong positive and intermediate type respectively. The 'p' value being 0.7920 ($p>0.05$), the result was statistically insignificant.

Table 10: Association of EGFR Staining intensity with site of tumour

Site of tumour	Negative	%	Weak positive	%	Intermediate	%	Strong positive	%	Total	%	χ^2	p-value
Right upper lobe	2	10.00	1	5.00	9	45.00	8	40.00	20	50.00	9.8120	0.3660
Right lower lobe	2	22.22	1	11.11	4	44.44	2	22.22	9	22.50		
Left upper lobe	2	40.00	1	20.00	0	0.00	2	40.00	5	12.50		
Left lower lobe	0	0.00	1	16.67	1	16.67	4	66.67	6	15.00		
Total	6	15.00	4	10.00	14	35.00	16	40.00	40	100.00		

Out of the total cases distributed in the two lungs at different sites, most of them showed intermediate to strong type of positivity for EGFR staining intensity.

Majority of the cases, 4/6(66.67%) in left lower lobe and 9/20(45%) in the right upper lobe showed strong positive and intermediate type of staining intensity respectively.

The 'p' value being 0.3660 ($p>0.05$), the result is statistically insignificant.

Graph 6: Association of EGFR Staining intensity with site of tumour

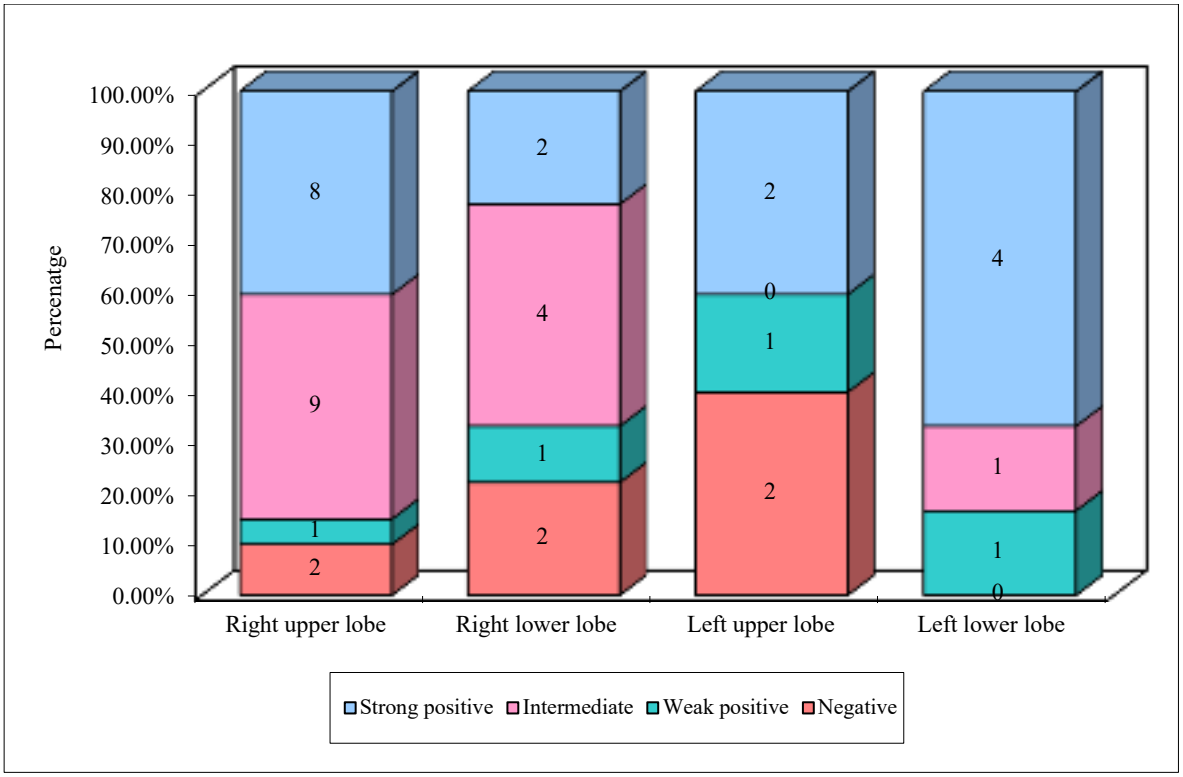


Table 11: Association of EGFR Staining intensity with types of tumours

Type of carcinoma	Negative	%	Weak positive	%	Intermediate	%	Strong positive	%	Total	%	χ^2	p-value
Adenocarcinoma	2	9.52	3	14.29	8	38.10	8	38.10	21	52.50	29.5930	0.0420*
Squamous cell carcinoma	0	0.00	1	7.69	5	38.46	7	53.85	13	32.50		
Adenocarcinoma -Lepidic variant	0	0.00	0	0.00	1	100.0	0	0.00	1	2.50		
Squamous cell carcinoma-keratinizing variant	0	0.00	0	0.00	0	0.00	1	100.0	1	2.50		
Sarcoma	1	100.	0	0.00	0	0.00	0	0.00	1	2.50		
Small cell carcinoma	1	100.0	0	0.00	0	0.00	0	0.00	1	2.50		
spindle cell neoplasm	2	100.	0	0.00	0	0.00	0	0.00	2	5.00		
Total	6	15.0	4	10.00	14	35.00	16	40.00	40	100.0		

*$p<0.05$

Intermediate to strong type of staining intensity was shown by most of the adenocarcinomas and squamous cell carcinomas. The only case of lepidic variant of adenocarcinoma and keratinizing variant of squamous cell carcinoma in this study showed intermediate and strong positive staining intensity respectively.

All the other tumours reported in the study like sarcoma, small cell carcinoma and spindle cell neoplasm showed negative staining intensity in 1/1(100%), 1/1(100%) and 2/2(100%)of cases respectively.

No case amongst squamous cell carcinomas and of its keratinizing variant too, showed negative staining intensity pattern associated with them. Percentage wise, the squamous cell carcinomas were reported to have maximum EGFR staining property followed by adenocarcinomas. The 'p' value was 0.0420 ($p<0.05$). The result was statistically significant.

Table 12: Association of EGFR Staining intensity with smoking status, diet, family history

	Negative	%	Weak positive	%	Intermediate	%	Strong positive	%	Total	%	χ^2	p-value
Smoking status												
Yes	4	15.38	1	3.85	9	34.62	12	46.15	26	65.00	3.5270	0.3170
No	2	14.29	3	21.43	5	35.71	4	28.57	14	35.00		
Diet												
Vegetarian	2	10.53	4	21.05	9	47.37	4	21.05	19	47.50	9.7340	0.0210*
Mixed	4	19.05	0	0.00	5	23.81	12	57.14	21	52.50		
Family history												
Present	0	0.00	0	0.00	2	33.33	4	66.67	6	15.00	3.0250	0.3880
Absent	6	17.65	4	11.76	12	35.29	12	35.29	34	85.00		
Total	6	15.00	4	10.00	14	35.00	16	40.00	40	100.0		

*$p<0.05$

Majority of 12/26(46.15%) cases with positive smoking status showed strong positive type of staining intensity. 4/26(15.38%)cases with positive smoking status also showed negative type of staining intensity. No association was found between these two parameters. The 'p' value was 0.3170($p>0.05$) and the result was statistically insignificant.

12/21(57.14%)patients on mixed diet, were reported to have strong positive type of staining intensity. The 'p' value was 0.0210 ($p<0.05$), thus making the result statistically significant.

Majority of 4/6(66.67%) cases with positive family history showed strong positive type of staining intensity. But, majority of 12/34(35.29%) cases with negative family for lung cancer too, showed strong positive type of staining intensity. No association was found between these two parameters. The 'p' value was 0.3880($p>0.05$) and the result was statistically insignificant.

Graph 7: Association of EGFR Staining intensity with smoking status, diet and family history

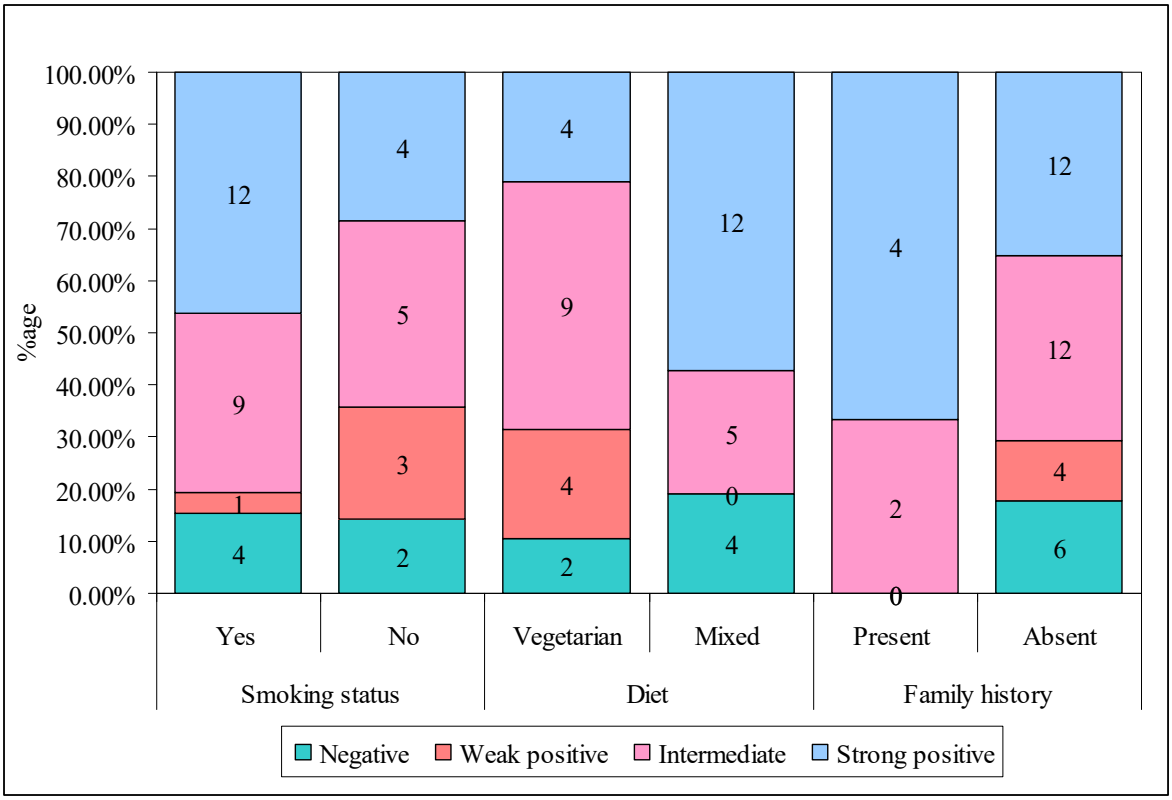


Table 13: Association of EGFR Staining intensity with symptoms

Symptoms	Negative	%	Weak positive	%	Interme diate	%	Strong positive	%	Total	%	χ^2	p-value
Cough												
Present	3	8.57	4	11.43	12	34.29	16	45.71	35	87.50	10.6120	0.0141*
Absent	3	60.00	0	0.00	2	40.00	0	0.00	5	12.50		
Dyspnoea												
Present	1	4.00	2	8.00	10	40.00	12	48.00	25	62.50	7.1870	0.0660
Absent	5	33.33	2	13.33	4	26.67	4	26.67	15	37.50		
Loss of appetite												
Present	0	0.00	0	0.00	12	48.00	13	52.00	25	62.50	22.2860	0.0001*
Absent	6	40.00	4	26.67	2	13.33	3	20.00	15	37.50		
Weight loss												
Present	0	0.00	0	0.00	11	40.74	16	59.26	27	67.50	29.2550	0.0001*
Absent	6	46.15	4	30.77	3	23.08	0	0.00	13	32.50		
Hemoptysis												
Present	1	5.56	1	5.56	6	33.33	10	55.56	18	45.00	4.5980	0.2040
Absent	5	22.73	3	13.64	8	36.36	6	27.27	22	55.00		
Total	6	15.00	4	10.00	14	35.00	16	40.00	40	100.0		

*$p<0.05$

Majority of the cases having cough [16/35(45.71%)], dyspnoea[12/25(48%)], loss of appetite[13/25(52%)], weight loss[16/27(59.26%)] and hemoptysis[10/18(55.56%)] as symptoms, showed strong positive type of staining intensity by EGFR.

The ‘p’ values for parameters of cough, loss of appetite and weight loss were 0.0141, 0.0001 and 0.0001 respectively. The ‘p’ value was <0.05 in each of these tests. Thus, the results of these parameters were statistically significant. EGFR staining intensity thus showed an association with these symptoms.

The ‘p’ values for parameters of dyspnoea and hemoptysis were 0.0660 and 0.2040 respectively. The ‘p’ value was >0.05 in each of these tests, leading the respective results to be statistically insignificant.

Graph 8: Association of EGFR Staining intensity with symptoms

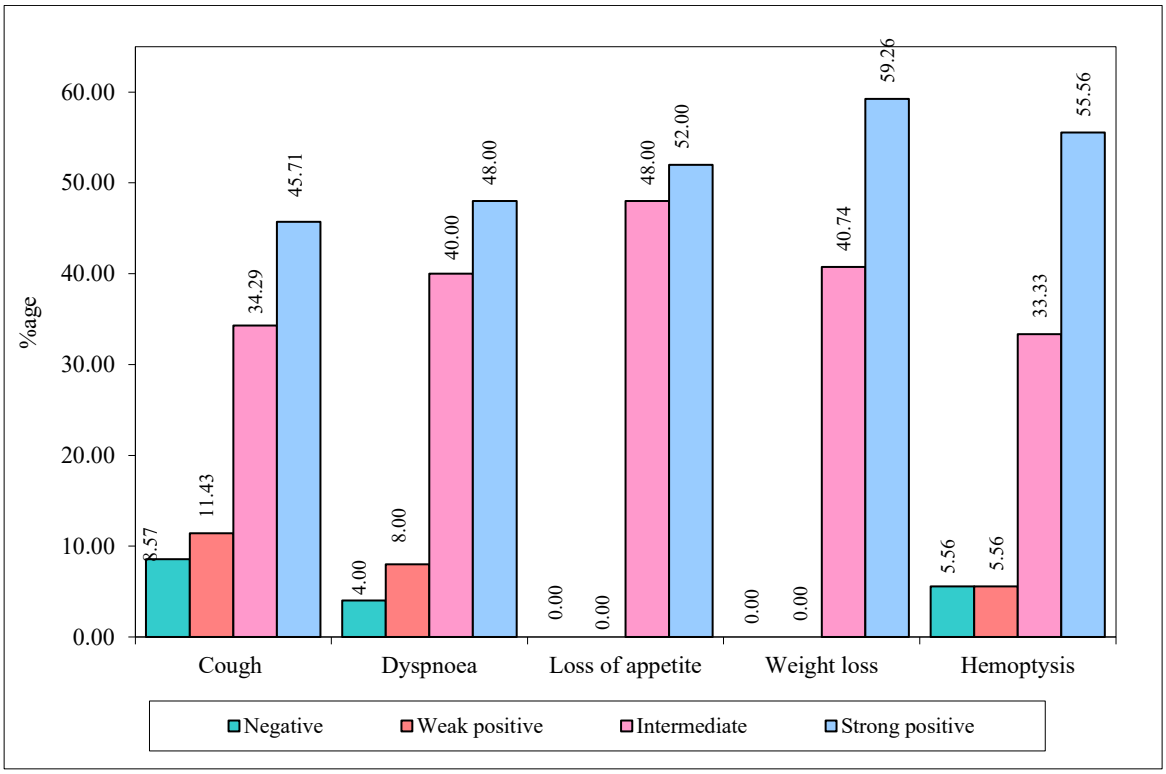
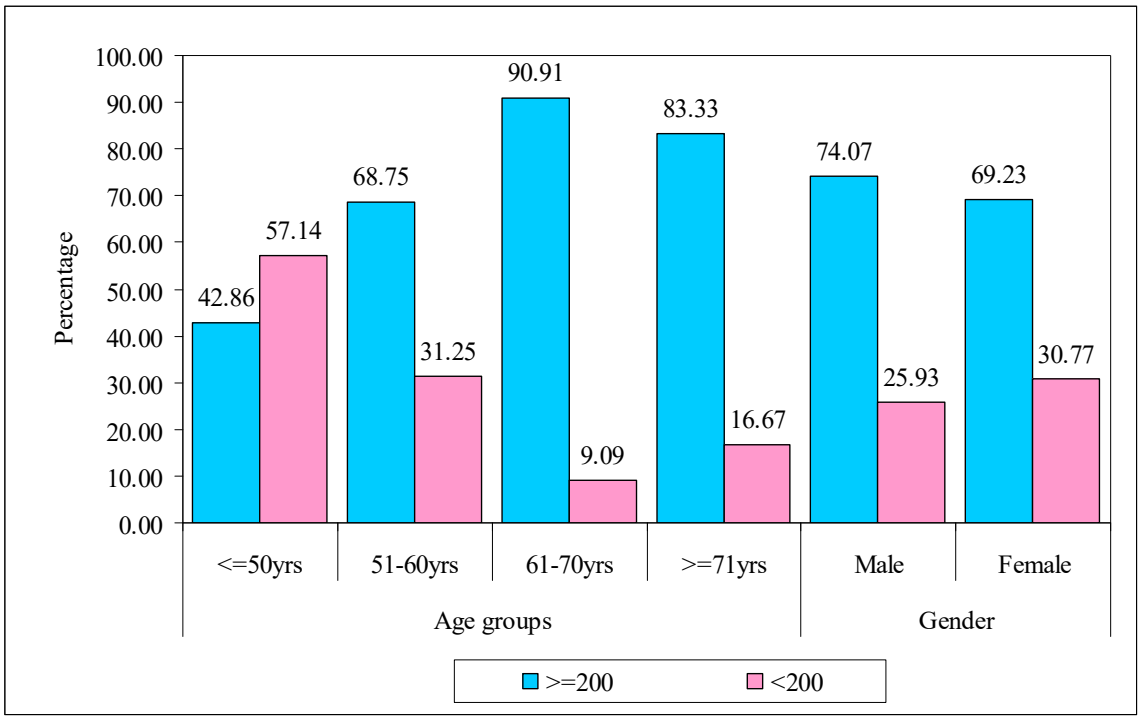


Table 14: Association between demographic profile and H-score

	>=200	%	<200	%	Total	%	χ^2	p-value
Age groups								
<=50yrs	3	42.86	4	57.14	7	17.50	5.4210	0.1430
51-60yrs	11	68.75	5	31.25	16	40.00		
61-70yrs	10	90.91	1	9.09	11	27.50		
>=71yrs	5	83.33	1	16.67	6	15.00		
Gender								
Male	20	74.07	7	25.93	27	67.50	0.1030	0.7480
Female	9	69.23	4	30.77	13	32.50		
Total	29	72.50	11	27.50	40	100.00		

Graph 9: Association between demographic profile and H-score

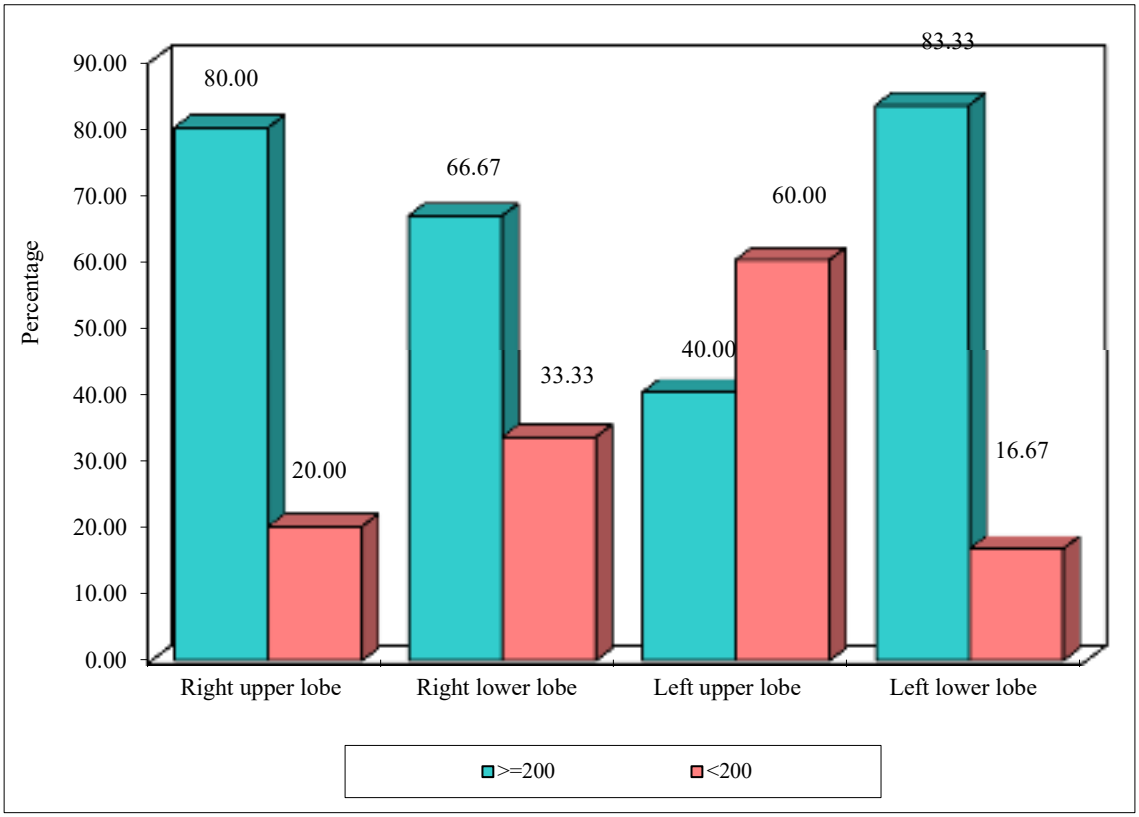


Out of the total cases distributed in different age groups, maximum percentage of cases showed IHC positive status with H-score >=200 as opposed to age group of <=50 yrs, where most of the cases were IHC negative with H-score <200. The 'p' value of 0.1430($p>0.05$) was calculated. The result was statistically insignificant. Considering the gender parameter for the statistical test, p value was 0.7480($p>0.05$). The test result was statistically insignificant.

Table 15: Association of H-score with site of tumour

Site of tumour	>=200	%	<200	%	Total	%	χ^2	p-value
Right upper lobe	16	80.00	4	20.00	20	50.00	3.7200	0.2930
Right lower lobe	6	66.67	3	33.33	9	22.50		
Left upper lobe	2	40.00	3	60.00	5	12.50		
Left lower lobe	5	83.33	1	16.67	6	15.00		
Total	29	72.50	11	27.50	40	100.00		

Graph 10: Association of H-score with site of tumour



Majority of tumours [5/6(83.33%)] located in lower lobe of left sided lung showed IHC positive status (H-score >=200). The 'p' value was 0.2930. The result was statistically insignificant.

Table 16: Association of H-score with types of tumours

Type of carcinoma	>=200	%	<200	%	Total	%	χ^2	p-value
Adenocarcinoma	15	71.43	6	28.57	21	52.50	13.8740	0.0310*
Squamous cell carcinoma	12	92.31	1	7.69	13	32.50		
Adenocarcinoma-Lepidic variant	1	100.00	0	0.00	1	2.50		
Squamous cell carcinoma-keratinizing variant	1	100.00	0	0.00	1	2.50		
Sarcoma	0	0.00	1	100.00	1	2.50		
Small cell carcinoma	0	0.00	1	100.00	1	2.50		
spindle cell neoplasm	0	0.00	2	100.00	2	5.00		
Total	29	72.50	11	27.50	40	100.00		

*p<0.05

H-score >=200(IHC positive) was shown by 15/21(71.43%)of adenocarcinomas and 12/13(92.31%)of squamous cell carcinomas. The only case of lepidic variant of adenocarcinoma and keratinizing variant of squamous cell carcinoma in this study were reported to have H-score>=200 (IHC positive) each.

Rest of the tumours viz., sarcoma, small cell carcinoma and spindle cell neoplasm showed H-score<200 (IHC negative) in 1/1(100%), 1/1(100%) and 2/2(100%)of cases respectively.

Percentage wise, most of the squamous cell carcinomas followed by adenocarcinomas showed to have H-score>=200(IHC positive). The ‘p’ value was 0.0310. The result was statistically significant.

Graph 11: Association of H-score with types of tumours

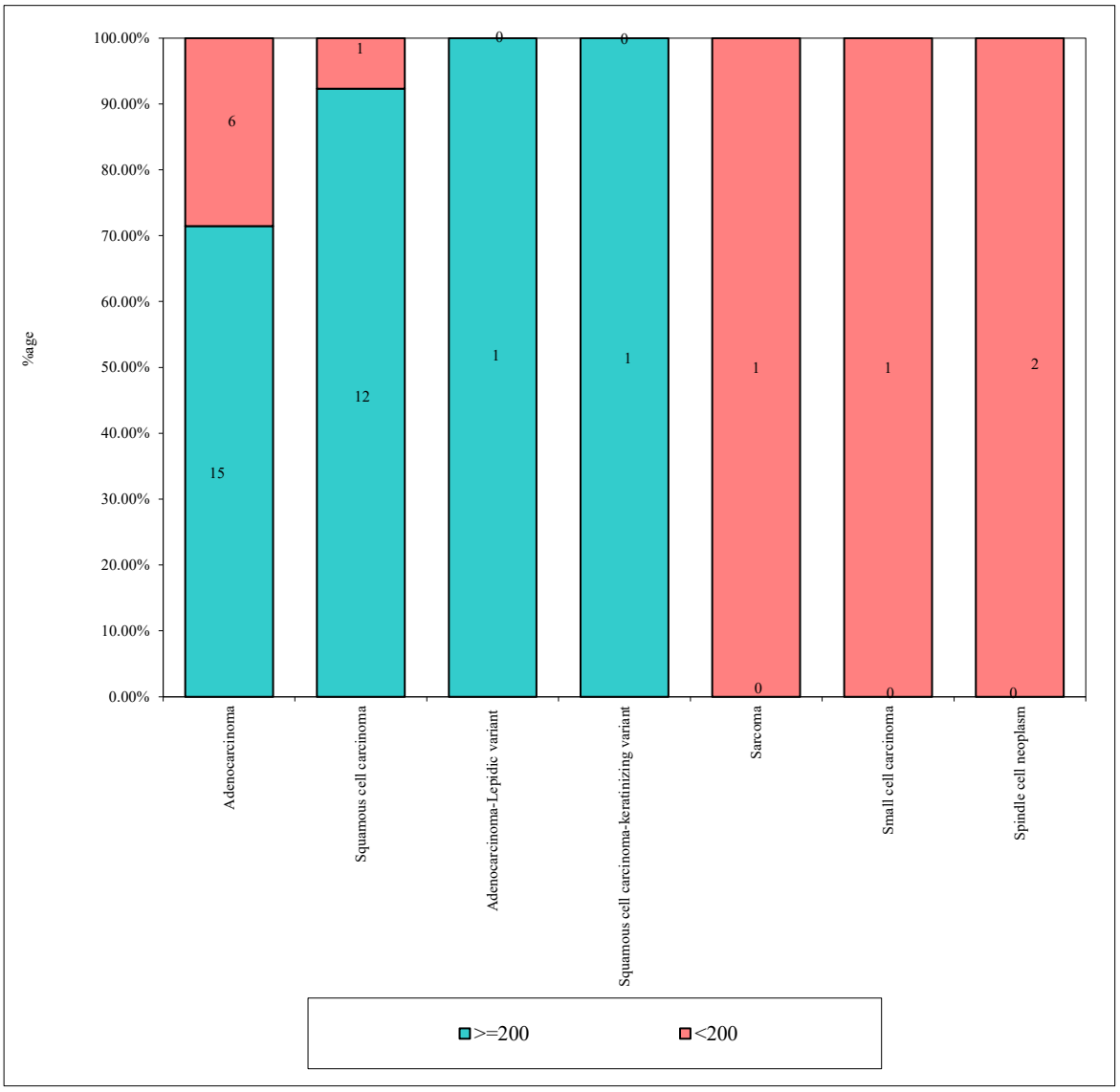


Table 17: Association of H-score with smoking status, diet and family history

	>=200	%	<200	%	Total	%	χ^2	p-value
Smoking status								
Yes	20	76.92	6	23.08	26	65.00	0.7290	0.3930
No	9	64.29	5	35.71	14	35.00		
Diet								
Vegetarian	12	63.16	7	36.84	19	47.50	1.5840	0.2080
Mixed	17	80.95	4	19.05	21	52.50		
Family history								
Present	6	100.00	0	0.00	6	15.00	2.6770	0.1020
Absent	23	67.65	11	32.35	34	85.00		
Total	29	72.50	11	27.50	40	100.00		

Majority of the cases with positive smoking status [20/26(76.92%)], mixed dietary intake [17/21(80.95%)] and positive family history [6/6(100%)] showed H-score >=200. The 'p' values for each of them was calculated to be p>0.05. The results for these parameters were statistically insignificant.

Graph 12: Association of H-score with smoking status, diet and family history

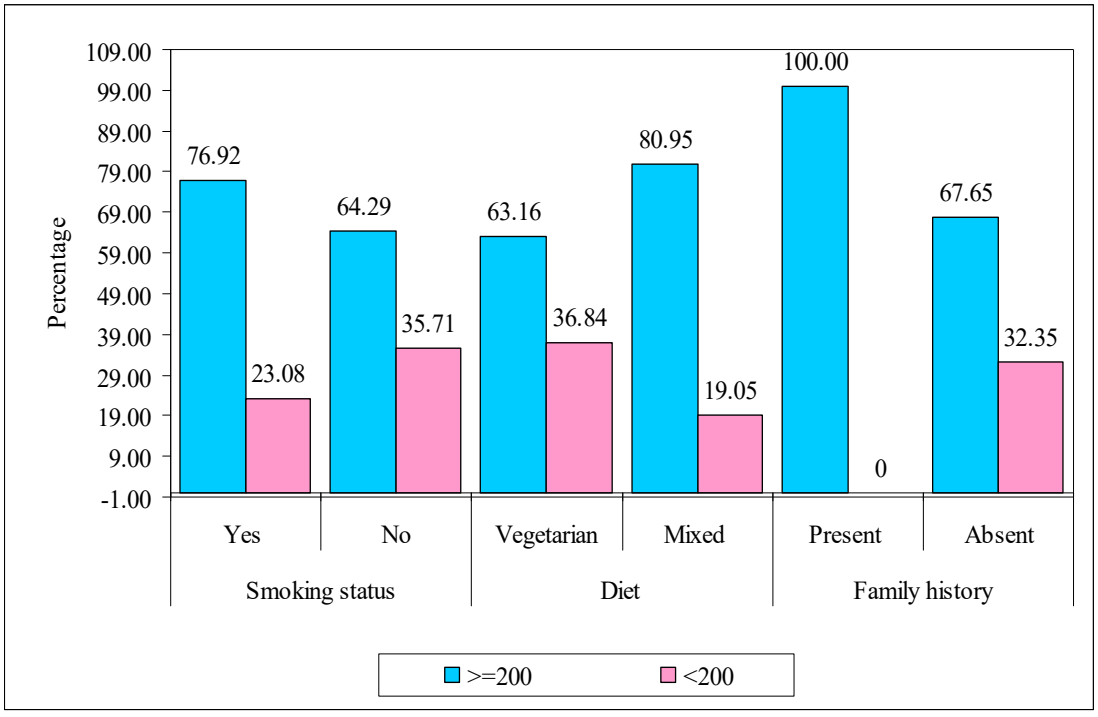
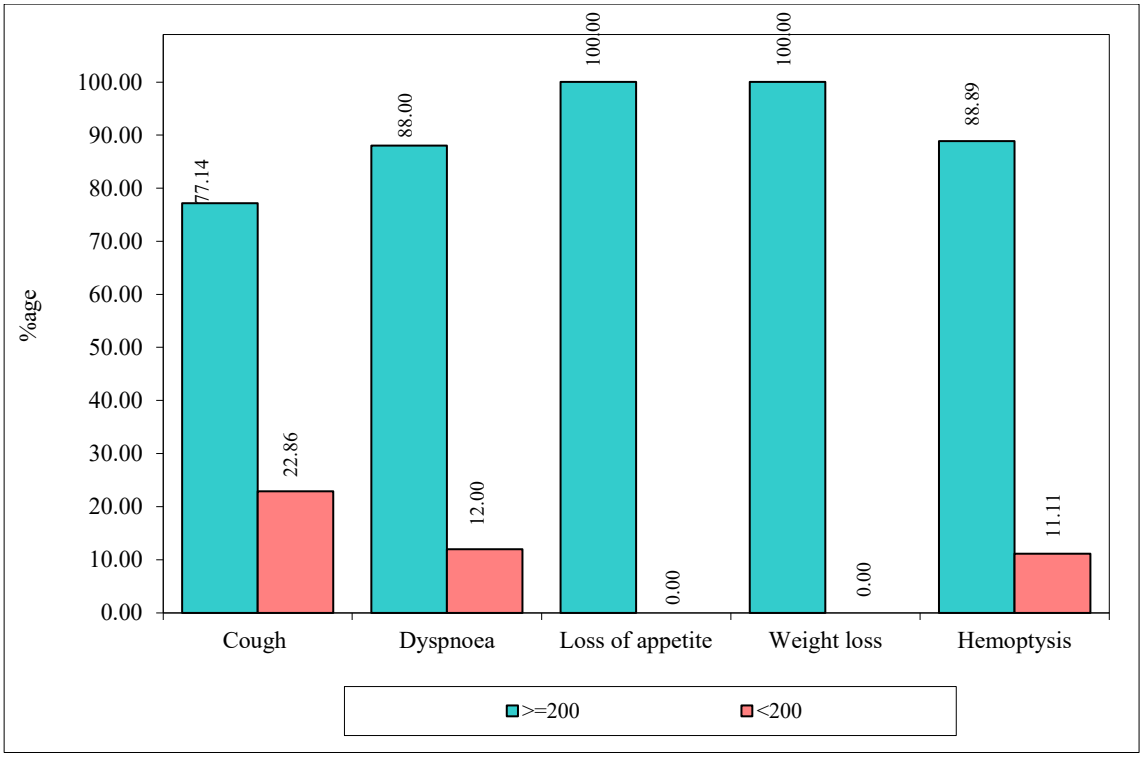


Table 18: Association of H-score with symptoms

	>=200	%	<200	%	Total	%	χ^2	p-value
Cough								
Present	27	77.14	8	22.86	35	87.50	3.0270	0.0820
Absent	2	40.00	3	60.00	5	12.50		
Dyspnoea								
Present	22	88.00	3	12.00	25	62.50	8.0330	0.0050*
Absent	7	46.67	8	53.33	15	37.50		
Loss of appetite								
Present	25	100.00	0	0.00	25	62.50	25.2870	0.0001*
Absent	4	26.67	11	73.33	15	37.50		
Weight loss								
Present	27	100.00	0	0.00	27	67.50	31.5120	0.0001*
Absent	2	15.38	11	84.62	13	32.50		
Hemoptysis								
Present	16	88.89	2	11.11	18	45.00	4.4090	0.0360*
Absent	13	59.09	9	40.91	22	55.00		
Total	29	72.50	11	27.50	40	100.00		

*p<0.05

Graph 13: Association of H-score with symptoms



Majority of the cases with cough [27/35(77.14%)], dyspnoea [22/25(88%)], loss of appetite [25/25(100%)], weight loss [27/27(100%)] and hemoptysis [16/18(88.89%)] showed H-score>=200 (IHC positive).

The 'p' values for association of H-score with cough, dyspnoea, loss of appetite, weight loss and hemoptysis were 0.0820, 0.0050, 0.0001, 0.0001 and 0.0360 respectively.

The 'p' value was 0.0820 and thus no significant association between cough and H-score could be established. The result was statistically insignificant.

Except for cough, a significant association can be established between H-score and the other symptoms as the results for them were statistically significant.

DISCUSSION

In an attempt to look for possible targets that can be exploited for prognostic, diagnostic or therapeutic use, various markers are being studied, one of which is EGFR. Few studies have been undertaken to understand the part played by EGFR in lung tumours and still fewer are performed in Indian settings.

In this study, along with the histopathological study of lung tumours, the association of EGFR expression with various clinicopathological parameters was studied by EGFR staining intensity and H-score methods.

Table 19: Number of cases in different studies

Study	Year(s) of study	Number of cases
Avilés-Salas et al.[60]	2008-2012	85
Douillard et al.[61]	2014	971
Hirsch et al.[62]	2008	1692
O'Byrne K et al.[63]	2004-2006	1125
Pirker R et al.[64]	2012	1121
J.Mazières et al.[65]	2005-2008	889
HIRSCH et al.[66]	1993-1999	183
Atkins D[67]	2000-2001	10(lung)/40
Mascaux et al.[68]	1995-2008	98
Marchetti et al.[69]	1998-2002	860
Kris M et al.[25]	2000-2001	221
Cheng et al.[57]	2012-2014	246
Kim J[59]	2006-2014	829
Present study	2019-2020	40

Table 20: Comparison of Age distribution

Study	Median/Mean Age (in years)
Kris M et al.[25]	Median =61 Range=(34-84)
Cheng et al.[57]	Median=57 Range=(22-78)
Kim J[59]	Median=66.5
Avilés-Salas et al.[60]	Median=44.7(for <60yrs) 55.3(for >60yrs) Mean age=61.76
Pirker R et al.[64]	Median=59(18-78)
J.Mazières et al.[65]	Median=60 (33-83)
Marchetti et al.[69]	Mean=62.7(32-85)
Present study	Median=60(21-86) Mean=58.90

In this study, the mean age of presentation was 58.90 years and median was 60 years which in concordance with the above studies.

Table 21: Comparison of Gender Distribution

Study	Males	Females
Cheng et al.[57]	24.4%	75.6%
Kim J[59]	64.5%	35.5%
Avilés-Salas et al.[60]	42.4%	57.6%
O'Byrne K et al.[63]	70%	30%
Pirker R et al.[64]	70%	30%
J.Mazières et al.[65]	73%	27%
HIRSCH et al.[66]	58%	42%
Marchetti et al.[69]	87%	13%
Present study	67.50%	32.50%

In the present study, 67.50% cases occured in males and 32.50% cases were seen in females. This is in league with the studies by Kim J[59] , O'Byrne K et al.[63] , Pirker R et al.[64] and J.Mazières et al.[65]

Table 22: Comparison of smoking status

Studies	Smokers	Non-smokers
O'Byrne K et al.[63]	78%	22%
Kim J[59]	66.9%	33.1%
Avilés-Salas et al.[60]	60%	40%
Pirker R et al.[64]	78%	22%
Marchetti et al.[69]	87%	13%
Present study	65%	35%

The current study reported 65% of smokers and 35% of non-smokers which correlates with the study by Kim J[59] and Avilés-Salas et al.[60]

Table 23: Comparison of percentages of types of lung tumours in various studies

Study	Percentage of types lung tumours (%)		
	Adenocarcinomas and variants	Squamous cell carcinomas and variants	Others
Kris M et al.[25]	69%	14%	17%
Cheng et al.[57]	92.3%	-	7.7%
Kim J[59]	64.8%	35.2%	
Avilés-Salas et al.[60]	77.6%	22.4%	
O'Byrne K et al.[63]	46%	34%	20%
J.Mazières et al.[65]	47%	38%	15%
HIRSCH et al.[66]	46%	49%	05%
Marchetti et al.[69]	44%	53%	03%
Present study	55%	35%	10%

In the present study, adenocarcinomas were reported to be the most common tumours, which correlates with many studies in the table and percentage wise, this study nearly corresponds to study percentages by J.Mazières et al.[65]. But due to lesser sample size of study as opposed to large number of cases in other studies, this percentage variation could be observed.

Table 24: Comparison between percentages of symptoms in various studies

Study / Symptoms	Kris M et al.[25]	PRESENT STUDY
Cough	42%	87.50%
Dyspnoea	59%	62.50%
Loss of appetite	53%	62.50%
Weight loss	43%	67.50%

This variation may be attributed to gefitinib treatment cycles received by few study subjects in the study by Kris M et al.[25]

Centrally located tumours are prone to cause these symptoms and especially hemoptysis[29] . This is in agreement to the present study as all the cases of squamous cell carcinomas in present study showed hemoptysis as a symptom.

An important conclusion that family history favours adenocarcinoma and females[57] was evident from the present study results.

Table 25: Comparison of EGFR expression percentages in various studies

Study	EGFR expression+ %(N=evaluated cases for EGFR)
Kim J[59]	37.2% (N=829)
Hirsch et al.[62]	69%(N=736)
Present study	85%(N=40)

N= sample size, + =present

In studies by Kim J[59] and Hirsch et al.[62], sample size was much higher compared to this study which explains the percentage variation. Other factors attributable for this fluctuation are the methods used to evaluate expression of this marker, definitions of criteria and different cut-off values in each study.

Comparison of H-scores in different studies

A study by HIRSCH et al[66], concluded that a majority of NSCLCs have high EGFR expression particularly squamous cell carcinomas and lepidic adenocarcinomas. This is in accordance with the present study.

In a study by Pirker et al[64], samples with reference scores H< 200 and >= 200 showed mean concordance rates of 94.7% and 85.6% respectively. This study lead to a conclusion that EGFR IHC expression is a potent marker to determine effectiveness of cetuximab drug in NSCLCs subjects. Cutoff value for H-score in the present study was 200, where <200 was reasoned IHC negative and >=200 was labelled as IHC positive. However, the present study did not aim to study the treatment protocols and their associations with EGFR expression.

In a study by Hirsch et al[66], a lower cutoff score for EGFR expression has a better ability to resolve positive and negative EGFR IHC results. This was seen in harmony with the present study.

In a study by Avilés-Salas et al.[60], near accurate concordance for H-scores was calculated by use of low cutoff value of 100(73.4-83.4%) as opposed to H-score with cutoff of 200 that showed a concordance of 67.5-77.3%.

Ultimately, the variation in the expression of EGFR in different studies could be attributed to the type of tissue sampling used. Other causes can be the method of analysis of data, threshold variation and most importantly the size of the sample studied.

CONCLUSION

In the present study, we have attempted to study histopathological types and immunohistochemical expression of EGFR in various lung tumours and its association with various parameters. EGFR was seen to be expressed relatively higher in sixth decade and preponderantly in male patients.

The EGFR expression was reported in majority of squamous cell carcinomas, adenocarcinomas and their variants by methods of estimating staining intensity of analyzable tumour cells and calculating H-score. There was a significant association between EGFR IHC expression and the types of lung tumours along with few clinicopathological parameters.

Squamous cell carcinomas and adenocarcinomas of lung, with or without regional lymph-node metastases, are difficult to treat effectively while maintaining the functions of vital structures. The final outcome of patients who present with advanced stage of the disease still remains poor.

EGFR is a prime target for anticancer therapy and is beneficial when EGFR inhibitor treatment is combined with radiotherapy to treat locally advanced cancers. Thus, the expression of EGFR protein can be used as a marker for targeted therapies in patients suffering from these lung tumours.

SUMMARY

Present study was a descriptive observational study of 40 cases from January 2019 to December 2020, and was performed in Pathology Department of KAHER's Jawaharlal Nehru Medical College and Dr Prabhakar Kore Hospital & Research Centre, Belagavi.

The aim of present study was to evaluate the expression of EGFR in lung tumours and to study various types of lung tumours and its subtypes.

Significant findings in this study were as follows:

1. Patients ranging from a minimum age of 21 years to a maximum of 86 years were present in the study. The mean age of subjects was 58.90 years with ±13.95 standard deviation. The median age of presentation was 60 years.

2. There was a clear male preponderance with male to female ratio of 2.08:1.

3. A majority, 40% of cases were present in 51-60 years age group.

4. Out of the total cases, 65% were smokers, 52.50% had mixed diet intake, 15% were positive for family history of lung cancer.

5. 50% of tumours in the study were located in the upper lobe of right lung.

6. Out of total cases, 52.50% were adenocarcinomas and 32.50% were squamous cell carcinomas. One case of lepidic variant of adenocarcinoma and keratinizing variant of SCC each was reported in this study. Other tumours reported were sarcoma, small cell carcinoma and spindle cell neoplasm.

7. 85% cases showed positive staining intensity for EGFR, among which 10% were weak positive, 35% were intermediate and 40% were strong positive.

8. 72.50% cases were EGFR IHC positive by H-score method.

9. Of the total symptoms included in this study, 87.50% had cough as the most common symptom, 62.50% had dyspnoea, 62.50 % showed loss of appetite, 67.50% presented with weight loss and 45% had hemoptysis as the least common symptom.

10. Increasing age and types of lung tumours like squamous cell carcinomas, adenocarcinomas and their variants, showed a significant association with intensity of EGFR staining($p<0.05$).

11. There was no significant association ($p>0.05$) between intensity of EGFR staining and parameters like gender, site of tumour, smoking status and family history.

12. A majority of squamous cell carcinomas followed by adenocarcinomas had H-score of >=200[IHC positive]. Thus a significant association ($p<0.05$) between H-score and types of lung tumours was established.

13. There was no significant association ($p>0.05$) between H-score and parameters like age group, gender, site of tumour, smoking status, diet intake, family history and cough.

LIMITATION

A major limitation of our study was smaller sample size. Hence, more studies with larger study population can be undertaken in future to establish a significant correlation among these entities.

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ANNEXURE I

INFORMED CONSENT

HISTOPATHOLOGICAL STUDY AND IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN LUNG TUMOURS - A HOSPITAL BASED STUDY AT KLES DR.PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI

Purpose of the study: The purpose of this study is to **KNOW ABOUT EXPRESSION OF A RECEPTOR KNOWN AS EPIDERMAL GROWTH FACTOR RECEPTOR(EGFR) IN OUR HUMAN BODY IN VARIOUS LUNG CANCERS BY THE HELP OF IMMUNOHISTOCHEMISTRY METHOD WHICH WILL FURTHER BENEFIT PATIENTS OF LUNG CANCERS FOR TARGETED THERAPEAUTIC TREATMENT.**

Procedure: During this study , you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge .If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know the prognosis for providing appropriate prevention and treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.

2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KLE University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following persons-

DR._____
Professor
Department Of Pathology,
J.N.Medical College, KAHER, Belagavi-590010

REG NO: BN0119009
Department Of Pathology,
J.N.Medical College, KAHER, Belagavi-590010

If you have any queries about your rights as a study subject, you may call Dr. Roopa Bellad, Professor, Department of Paediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No- 9448113403, at J.N. Medical College, Belagavi**.**

Name of the participant:
(signature /thumbprint)

Name of the witness :
(signature)

Name of the investigator:
(signature)

Date:
Address:
Phone no:

सूचित सहमति

हिस्टोपैथोलॉजिकल स्टडी और फेफड़े के ट्यूमर में एपिडर्मल ग्रोथ फैक्टर रिसेप्टर के इम्यूनोहिस्टोकेमिकल एक्सप्रेशन

अध्ययन क□उद्देश्य :

इस अध्ययन क□उद्देश्य एपिडर्मल ग्रोथ फैक्टर रिसेप्टर (ईजीएफआर) के रूप में हमारे मानव शरीर में विभिन्न फेफड़े के कैंसर में इम्यूनोहिस्टोकेमिस्ट्री पद्धति की मदद से ज्ञान करने के बारे में जानना□है, जो लक्षित थैरेपिक उपचार के लिए फेफड़े के कैंसर के रोगियों को और अधिक लाभान्वित करते हैं।

प्रक्रिय□:

इस अध्ययन के दौरान, आपसे इतिहास और पृष्ठभूमि के बारे में सवाल पूछे जाएंगे और आप अपने ज्ञान क□ सबसे अच्छा□जवाब देने वाले हैं,

यदि आप इस अध्ययन में खुद को नामांकित करने के लिए सहमत हैं, तो आपको अपने वर्तमान, अतीत और पारिवारिक इतिहास और आपकी नैदानिक अभिव्यक्तियों के बारे में साक्षात्कार दिया□ जाएगा□

जोखिम और लाभ :

इस अध्ययन में भाग लेने में कोई जोखिम नहीं हैं और लाभ यह है कि हम थायरॉइड कैंसर के निदान के लिए एक बेहतर तरीका□जान पाएंगे जो उचित उपचार प्रदान करने के लिए आवश्यक है.

विकल्प:

इस अध्ययन में भाग लेना□स्वैच्छिक है। आप इस अध्ययन में भाग नहीं□लेने का□चयन कर सकते हैं या□यदि आप अभी भाग लेने का□निर्णय लेते हैं, तो आप बाद में अपना□विचार बदल सकते हैं और अध्ययन से हट सकते हैं। अध्ययन चिकित्सक कभी भी इस अध्ययन में आपकी भागीदारी को समाप्त कर सकता□है।

गोपनीयत□

इस अध्ययन के दौरान आपके बारे में एकत्र की गई सभी जानकारी को कानून द्वारा□अनुमत सीमा□तक गोपनीय रख□जाएगा□ कोड नंबर इस शोध रिकॉर्ड में आपकी पहचान करेंगे। इस अध्ययन से जानकारी प्रकाशित की जाएगी लेकिन आपकी पहचान किसी भी प्रकाशन में गोपनीय रहेगी। आपके बारे में या□अनुसंधान के दौरान आपके द्वारा□ दी गई जानकारी के अलावा□आपकी लिखित अनुमति के बिना□अन्य के लिए खुलासा□नहीं□किया□जाएगा□

1. अपने अधिकारों और कल्याण की रक्षा□के लिए आपातकाल में।

2. यदि कानून द्वारा□आवश्यक हो।

भागीदारी के लिए वित्तीय प्रोत्साहन :

इस अध्ययन में भाग लेने के लिए आपको कोई उपहार / प्रोत्साहन नहीं दिया जाएगा।

परिणाम प्रकाशित करने के लिए प्राधिकरण :

इस अध्ययन के परिणामों को कहेर, बेलगावी को एमडी डिग्री, समीक्षा और प्रकाशन के पूरा होने की आवश्यकता के हिस्से के रूप में भेजा जाएगा।

सहमति कथन

मैं स्वेच्छा से नीचे हस्ताक्षर करके इस अध्ययन में भाग लेने के लिए सहमत हूं। मैं किसी भी समय वापस ले सकता हूं। मैं इस फॉर्म पर हस्ताक्षर करके कोई कानूनी अधिकार नहीं छोड़ रहा हूं। नीचे दिया गया मेरा हस्ताक्षर दर्शाता है कि मैंने पढ़ा है, या यह मुझे, इस पूरी सहमति के रूप में पढ़ा गया है और मेरे सभी सवालों के जवाब दिए हैं

अध्ययन के दौरान या भविष्य में प्रश्नों के मामले में आप निम्नलिखित व्यक्तियों से सपर्क कर सकते हैं–

डॉ. _____

प्रोफ़ेसर पैथॉलॉजी विभाग,
जे.एन. मेडिकल कॉलेज,

REG NO: BN0119009
पैथॉलॉजी विभाग,
जे.एन. मेडिकल कॉलेज,

यदि आपके पास अध्ययन के विषय के रूप में आपके अधिकारों के बारे में कोई प्रश्न हैं, तो आप डॉ। रूपा बेल्लद, प्रोफेसर, बाल रोग विभाग, जेएन मेडिकल कॉलेज संस्थागत नैतिक अनुसंधान मानव विषय अनुसंधान समिति, अध्यक्ष फोन नंबर - 9448113403.

प्रतिभागी का नाम: (हस्ताक्षर / अंगूठे का निशान)

गवाह का नाम: (हस्ताक्षर / अंगूठे का निशान)

अन्वेषक का नाम: (हस्ताक्षर)

दिनांक:

ತಿಳುವಳಿಕೆಯುಳ್ಳ ಒಪ್ಪಿಗೆ

ಹಿಸ್ಟೊಪಾಥೋಲಾಜಿಕಲ್ ಸ್ಟಡಿ ಮತ್ತು ಶ್ವಾಸಕೋಶದ ಗೆಡ್ಡೆಗಳಲ್ಲಿ ಎಪಿಡರ್ಮಲ್ ಗ್ರೋತ್ ಫ್ಯಾಕ್ಟರ್ ರಿಸೆಪ್ಟರ್ ಇಮ್ಯುನೊಹಿಸ್ಟೋಕೆಮಿಕಲ್ ಅಭಿವ್ಯಕ್ತಿ

ಅಧ್ಯಯನದ ಉದ್ದೇಶ

ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶವೆಂದರೆ ನಮ್ಮ ಮಾನವ ದೇಹದಲ್ಲಿ ಎಪಿಡರ್ಮಲ್ ಗ್ರೋತ್ ಫ್ಯಾಕ್ಟರ್ ರಿಸೆಪ್ಟರ್ (ಇಜಿಎಫ್ಆರ್) ಎಂದು ಕರೆಯಲ್ಪಡುವ ರಿಸೆಪ್ಟರ್ ಅನ್ನು ವಿವಿಧ ಶ್ವಾಸಕೋಶದ ಕ್ಯಾನ್ಸರ್ಗಳಲ್ಲಿ ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿ ವಿಧಾನದ ಸಹಾಯದಿಂದ ತಿಳಿದುಕೊಳ್ಳುವುದು, ಇದು ಶ್ವಾಸಕೋಶದ ಕ್ಯಾನ್ಸರ್ ರೋಗಿಗಳಿಗೆ ಉದ್ದೇಶಿತ ಚಿಕಿತ್ಸಕ ಚಿಕಿತ್ಸೆಗಾಗಿ ಮತ್ತಷ್ಟು ಪ್ರಯೋಜನವನ್ನು ನೀಡುತ್ತದೆ.

ವಿಧಾನ:

ಈ ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ, ನಿಮಗೆ ಇತಿಹಾಸ ಮತ್ತು ಹಿನ್ನೆಲೆ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲಾಗುತ್ತದೆ ಮತ್ತು ನಿಮ್ಮ ಜ್ಞಾನದ ಅತ್ಯುತ್ತಮ ಉತ್ತರವನ್ನು ನೀವು ನೀಡಬೇಕಾಗುತ್ತದೆ,

ಈ ಅಧ್ಯಯನಕ್ಕೆ ನಿಮ್ಮನ್ನು ಸೇರಿಸಲು ನೀವು ಒಪ್ಪಿದರೆ, ನಿಮ್ಮ ಪ್ರಸ್ತುತ, ಹಿಂದಿನ ಮತ್ತು ಕುಟುಂಬದ ಇತಿಹಾಸ ಮತ್ತು ನಿಮ್ಮ ಕ್ಲಿನಿಕಲ್ ಅಭಿವ್ಯಕ್ತಿಗಳ ಬಗ್ಗೆ ನಿಮ್ಮನ್ನು ಸಂದರ್ಶಿಸಲಾಗುತ್ತದೆ.

ಅಪಾಯ ಮತ್ತು ಪ್ರಯೋಜನಗಳು

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವಲ್ಲಿ ಯಾವುದೇ ಅಪಾಯಗಳಿಲ್ಲ ಮತ್ತು ಸೂಕ್ತ ಚಿಕಿತ್ಸೆಯನ್ನು ಒದಗಿಸಲು ಅಗತ್ಯವಾದ ಥೈರಾಯ್ಡ್ ಕ್ಯಾನ್ಸರ್ ಅನ್ನು ಪತ್ತೆಹಚ್ಚಲು ಉತ್ತಮ ಮಾರ್ಗವನ್ನು ನಾವು ತಿಳಿದುಕೊಳ್ಳಲು ಸಾಧ್ಯವಾಗುತ್ತದೆ.

ಪರ್ಯಾಯಗಳು:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದು ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸದಿರಲು ನೀವು ಆಯ್ಕೆ ಮಾಡಬಹುದು ಅಥವಾ ನೀವು ಈಗ ಭಾಗವಹಿಸಲು ನಿರ್ಧರಿಸಿದರೆ, ನೀವು ನಂತರ ನಿಮ್ಮ ಮನಸ್ಸನ್ನು ಬದಲಾಯಿಸಬಹುದು ಮತ್ತು ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಬಹುದು. ಅಧ್ಯಯನದ ವೈದ್ಯರು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆಯನ್ನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಕೊನೆಗೊಳಿಸಬಹುದು.

ಗೌಪ್ಯತೆ

ಈ ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ನಿಮ್ಮ ಬಗ್ಗೆ ಸಂಗ್ರಹಿಸಲಾದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಕಾನೂನಿನಿಂದ ಅನುಮತಿಸುವ ಮಟ್ಟಿಗೆ ಗೌಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ. ಈ ಸಂಶೋಧನಾ ದಾಖಲೆಯಲ್ಲಿ ಕೋಡ್ ಸಂಖ್ಯೆಗಳು ನಿಮ್ಮನ್ನು ಗುರುತಿಸುತ್ತವೆ. ಈ ಅಧ್ಯಯನದ ಮಾಹಿತಿಯನ್ನು ಪ್ರಕಟಿಸಲಾಗುವುದು ಆದರೆ ಯಾವುದೇ ಪ್ರಕಟಣೆಯಲ್ಲಿ ನಿಮ್ಮ ಗುರುತು ಗೌಪ್ಯವಾಗಿರುತ್ತದೆ. ನಿಮ್ಮ ಲಿಖಿತ ಅನುಮತಿಯಿಲ್ಲದೆ ನಿಮ್ಮ ಬಗ್ಗೆ ಯಾವುದೇ ಮಾಹಿತಿ ಅಥವಾ ಸಂಶೋಧನೆಯ ಸಮಯದಲ್ಲಿ ನೀವು ಒದಗಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಇತರರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ:

1. ನಿಮ್ಮ ಹಕ್ಕುಗಳು ಮತ್ತು ಕಲ್ಯಾಣವನ್ನು ರಕ್ಷಿಸಲು ತುರ್ತು ಪರಿಸ್ಥಿತಿಯಲ್ಲಿ.

2. ಕಾನೂನಿನ ಪ್ರಕಾರ ಅಗತ್ಯವಿದ್ದರೆ.

ಭಾಗವಹಿಸುವಿಕೆಗೆ ಆರ್ಥಿಕ ಪ್ರೋತ್ಸಾಹ:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮಗೆ ಯಾವುದೇ ಉಡುಗೊರೆ / ಪ್ರೋತ್ಸಾಹ ಧನ ನೀಡಲಾಗುವುದಿಲ್ಲ.

ಫಲಿತಾಂಶಗಳನ್ನು ಪ್ರಕಟಿಸಲು ಅಧಿಕಾರ:

ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳನ್ನು ಎಂಡಿ ಪದವಿ, ವಿಮರ್ಶೆ ಮತ್ತು ಪ್ರಕಟಣೆಯ ಪೂರ್ಣಗೊಳಿಸುವಿಕೆಯ ಅವಶ್ಯಕತೆಯ ಭಾಗವಾಗಿ ಬೆಳಗವಿಯ ಕಾಹೇರ್‌ಗೆ ರವಾನಿಸಲಾಗುತ್ತದೆ.

ಒಪ್ಪಿಗೆಯ ಹೇಳಿಕೆ

ಕೆಳಗೆ ಸಹಿ ಮಾಡುವ ಮೂಲಕ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಾನು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪುತ್ತೇನೆ. ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂತೆಗೆದುಕೊಳ್ಳಬಹುದು. ಈ ಫಾರ್ಮ್ಗೆ ಸಹಿ ಮಾಡುವ ಮೂಲಕ ನಾನು ಯಾವುದೇ ಕಾನೂನು ಹಕ್ಕುಗಳನ್ನು ಬಿಟ್ಟುಕೊಡುತ್ತಿಲ್ಲ. ಕೆಳಗಿನ ನನ್ನ ಸಹಿ ನಾನು ಓದಿದ್ದೇನೆ, ಅಥವಾ ಅದನ್ನು ನನಗೆ ಓದಿದೆ, ಈ ಸಂಪೂರ್ಣ ಒಪ್ಪಿಗೆಯ ರೂಪ ಮತ್ತು ನನ್ನ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸಿದೆ ಎಂದು ಸೂಚಿಸುತ್ತದೆ

ಡಾ. _____

ಪ್ರೊಫೆಸರ್

ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ,

ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯ ಕಾಲೇಜು,

REG NO: BN0119009

ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ,

ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯ ಕಾಲೇಜು,

ಅಧ್ಯಯನದ ವಿಷಯವಾಗಿ ನಿಮ್ಮ ಹಕ್ಕುಗಳ ಬಗ್ಗೆ ನೀವು ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ, ನೀವು ಪೀಡಿಯಾಟ್ರಿಕ್ಸ್ ವಿಭಾಗದ ಪ್ರಾಧ್ಯಾಪಕ ಡಾ.ರೂಪಾ ಬೆಲ್ಲದ ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯ ಕಾಲೇಜು ಸಾಂಸ್ಥಿಕ ನೈತಿಕ ಸಮಿತಿಯ ಮಾನವ ವಿಷಯಗಳ ಸಂಶೋಧನೆ, ಅವರನ್ನು ಕರೆ ಮಾಡಬಹುದು, ಪಿಎಚ್ ಸಂಖ್ಯೆ- 9448113403, ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ಬೆಳಗವಿ

ಭಾಗವಹಿಸುವವರ ಹೆಸರು: (ಸಹಿ / ಹೆಬ್ಬೆರಳು)

ಸಾಕ್ಷಿಯ ಹೆಸರು: (ಸಹಿ / ಹೆಬ್ಬೆರಳು)

ತನಿಖಾಧಿಕಾರಿಯ ಹೆಸರು: (ಸಹಿ)

ದಿನಾಂಕ:

मा□हितीपूर्ण सम्मती

फुफ्फुसातील ट्यूमरमध्ये एपिडर्मल ग्रोथ फॅक्टर रिसेप्टरचा हिस्टोपॅथोलॉजिकल स्टडी आणि इम्यूनोहिस्टोकेमिकल एक्सप्रेशन

अभ्यासाचा उद्देश

या अभ्यासाचे उद्दीष्ट म्हणजे एपिडर्मल ग्रोथ फॅक्टर रिसेप्टर (ईजीएफआर) म्हणून ओळखल्या जाणाऱ्या रिसेप्टरच्या अभिव्यक्तीबद्दल जाणून घेणे इम्यूनोहिस्टोकेमिस्ट्री पद्धतीच्या सहाय्याने विविध फुफ्फुसाद्वारे कर्करोगाद्वारे आपल्या मानवी शरीरास लक्ष्यित थेरपीओटिक उपचारांसाठी फुफ्फुसाद्वारे कर्करोगाच्या रुग्णांना अधिक फायदा होईल.

प्रक्रिया:

या अभ्यासादरम्यान, आपणास इतिहास आणि पार्श्वभूमीसंबंधित प्रश्न विचारले जातील आणि आपल्यास आपल्या सर्वोत्तम ज्ञानाचे उत्तर द्यावे लागेल,

आपण या अभ्यासामध्ये स्वत: ची नावनोंदणी करण्यास सहमती दर्शविल्यास आपल्यास आपल्या वर्तमान, भूतकाळातील आणि कौटुंबिक इतिहासाबद्दल आणि आपल्या क्लिनिकल अभिव्यक्त्याविषयी मुलाखत दिली जाईल.

जोखीम आणि फायदे

या अभ्यासामध्ये भाग घेण्यास कोणताही धोका नाही आणि त्याचा फायदा म्हणजे आम्हास थायरॉईड कर्करोगाचे निदान करण्याचा एक चांगला मार्ग मोहित होऊ शकेल जो योग्य उपचार देण्यासाठी आवश्यक आहे.

विकल्प:

या अभ्यासामध्ये भाग घेणे ऐच्छिक आहे. आपण या अभ्यासामध्ये भाग न घेण्याची निवड करू शकता किंवा आपण आता भाग घेण्याचा निर्णय घेतल्यास आपण नंतर आपले मत बदलू आणि अभ्यासापासून दूर जाऊ शकता अभ्यास डॉक्टर या अभ्यासामधील आपला सहभाग कधीही रद्द करू शकतो.

गोपनीयता

या अभ्यासाच्या दरम्यान आपल्याबद्दल संकलित केलेली सर्व माहिती कायद्याद्वारे परवानगी असलेल्या मर्यादेपर्यंत गोपनीय ठेवली जाईल. कोड नंबर आपल्याला या संशोधन रेकॉर्डमध्ये ओळखतील. या अभ्यासाची माहिती प्रकाशित केली जाईल परंतु आपली ओळख कोणत्याही प्रकाशनास गोपनीय असेल. आपल्याबद्दल किंवा संशोधनादरम्यान प्रदान केलेली माहिती किंवा इतर माहिती आपल्या लिखित परवानगीशिवाय इतरांना उघड केली जाणार नाही:

1. आपत्कालीन परिस्थितीत आपले हक्क आणि कल्याण संरक्षित करण्यासाठी.

2. कायद्याने आवश्यक असल्यास.

सहभागासाठी आर्थिक प्रोत्साहन:

या अभ्यासामध्ये भाग घेण्यासाठी आपल्याला कोणतीही भेट / प्रोत्साहन दिले जाणार नाही.

परिणाम प्रकाशित करण्यासाठी अधिकृतता:

या अभ्यासाचे निकाल एमएडी पदवी, आढावा आणि प्रकाशन पूर्ण करण्याच्या आवश्यकतेचा भाग म्हणून कार्यालय, बेलगावीकडे पाठविला जाईल.

संमती विधान

मी खाली स्वाक्षरी करून या अभ्यासात भाग घेण्यास स्वेच्छेने सहमत आहे. मी केव्हाही माघार घेऊ शकतो. या फॉर्मवर सही करून मी कोणतेही कायदेशीर हक्क सोडत नाही. खाली माझी स्वाक्षरी सूचित करते की मी हा संपूर्ण संमती फॉर्म वाचला आहे किंवा मला वाचला गेला आहे आणि माझ्या सर्व प्रश्नांची उत्तरे दिली आहेत

डॉ. _____

प्रोफेसर
पॅथॉलॉजी विभाग,
जे.एन. मेडिकल कॉलेज,

REG NO: BN0119009

पॅथॉलॉजी विभाग,

जे.एन. मेडिकल कॉलेज,

आपल्याकडे अभ्यासाचा विषय म्हणून आपल्या हक्कांबद्दल काही शंका असल्यास आपण डॉ. रूपा बेल्लद, प्रोफेसर, बाल रोगशास्त्र विभाग, मानवी विषय संशोधनाची संस्था नैतिक समिती, अध्यक्ष जे.एन. मेडिकल कॉलेज बेलगावी. कॉल करू शकता फोन नंबर 9448113403.

सहभागीचे नाव: (स्वाक्षरी / अंगठ्याचा ठसा)

साक्षीदाराचे नाव: (सही / अंगठ्याचा ठसा)

चौकशीचे नाव: (स्वाक्षरी)

तारीख:

ANNEXURE II

PROFORMA

NAME:

AGE:

GENDER:

IP NO:

BIOPSY NO:

DATE OF COLLECTION:

CLINICAL DETAILS:

SMOKING STATUS: YES ?/NO?

DIET: VEG/NON VEG

WEIGHT (H/O RECENT WEIGHT LOSS):

PHYSICAL ACTIVITY:

PERSONAL HISTORY:

PAST HISTORY:

OTHERS:

H/O PREVIOUS SURGERY

FAMILY HISTORY

H/O MEDICAL ILLNESS

EXAMINATION:

SITE

SIDE

SIZE

INVESTIGATIONS:

OTHER INVESTIGATIONS:

CLINICAL STAGING:

TREATMENT ADVISED:

HORMONAL

RADIOTHERAPY

CHEMOTHERAPY

HISTOPATHOLOGICAL CLASSIFICATION:

EXPRESSION OF EGFR IN VARIOUS TUMOURS OF LUNG.

ANNEXURE III

ETHICAL CLEARANCE CERTIFICATE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed to be University)
Accredited 'A' Grade by NAAC (3rd Cycle) Placed in Category-1 by MHRD of India

JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: http://www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+91-0831) Office : 2472550
Principal: 2471701
Fax No. +91-0831 - 2420759

Ref: MDC/DOME/ 2261 **Date: 24/12/2019**

To
REG NO: BN0119009
PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"HISTOPATHOLOGICAL STUDY AND IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN LUNG TUMOURS- A TERTIARY HOSPITAL BASED STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Belludi)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANNEXURE IV

HEMATOXYLIN AND EOSIN STAINING

Bancroft D, Layton C. The haematoxylins and eosin. In:Kim SS,Bancroft D, Layton C (Eds). Bancroft's Theory and Practice of Histopathological Techniques. 8th Ed. China: Elsevier; 2019. p126-138.

1. Deparaffinize in Xylene I and II and III changes. (III change use warmed xylene) (5 minutes in each)

2. Rehydrate using

 Absolute ethanol 100% (5 minutes)

 Absolute Ethanol 100% (5 minutes)

3. Rinse in distilled water (5 minutes)

4. Rinse in running tap water (5 minutes)

5. Stain in Harris's haematoxylin by progressive method (2 minutes) Fresh and filtered

6. Rinse in running tap water (20 minutes)

7. Decolorize in 1% acid alcohol (1 second)

8. Rinse well in tap water (5 minutes)

9. Immerse in hot water bath, 55˚C for blueing (3 seconds)

10. Rinse in tap water (5 minutes)

11. Counterstain in Eosin (15 seconds)

12. Dehydrate with absolute alcohol 100% (2-4 dips)

13. Clear in xylene I and II (5 minutes)

14. Mount with DPX.

Harris Hematoxylin

1. Hematoxylin.....5g

2. Absolute ethanol.....50ml

3. Ammonium aluminum sulfate.....100g

4. Distilled water.....1000ml

5. Sodium iodate.....0.37g

Eosin solution

1 Yellow eosin = 1 gm

2 Distilled water = 80 ml

3 Ethanol = 320 ml

Glacial Acetic Acid = 2 drops

0.3% Acid Alcohol (0.3% HCl in 70% ethanol)

Stock solution – Eosin:

Stock – 1% aqueous Eosin – Y

Stock – 1% aqueous Phloxin B

Working Solution – Eosin:

100ml stock Eosin

10 ml stock Phloxin B

780 ml 95% Ethanol

4 ml glacial acetic acid

Working Solution – Hematoxylin:

Harris Hematoxylin, 1 litre

Working solution – 0.25% Acid alcohol

95% Ethanol, 2578 ml

dH2O, 950 ml

HCl, 9ml

Result: Nuclei – Blue, cytoplasm – Pink, RBCs – Red.

ANNEXURE V
IHC STAINING

IHC MARKER USED FOR THE STUDY: BIO SB EGFR

Clone: 31G7 Mouse Monoclonal

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations.

Immunogen : EGFR derived from A-431 cells.

Summary and Explanation: Epidermal Growth Factor Receptor (EGFR) is the receptor for epidermal growth factor (EGF). It is a member of the ErbB family receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER-2 neu (ErbB-2), HER-3 (ErbB-3) and HER-4 (ErbB-4).

Control : Skin, Placenta, Testis, Tonsil, Pancreas, Squamous Cell Carcinoma

Application : Breast Cancer, Colon & Gastrointestinal Cancer, Lung Cancer

Presentation: Anti-EGFR is a Mouse monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Procedure for IHC staining for EGFR marker :

Prepare formalin-fixed, paraffin-embedded tissue sections (Step 1-8):

1. Fix freshly dissected tissue (<3mm thick) with 2% paraformaldehyde from 1h to overnight at room temperature.
2. Rinse the tissue with running tap water for 5 min.

3. Dehydrate the tissue through 70%, 80%, 95% alcohol, 5 min each, followed with 3 times of 100% alcohol, 1 hours each.

4. Cleared the tissue in xylene for 2 times, 1 hours each.

5. Immerse the tissue in paraffin for 2 times, 1 hours each.

6. Embed the tissue in a paraffin block. The paraffin tissue block can be stored at room temperature for years.

7. Section the paraffin-embedded tissue block at 3-4 µm thickness on a microtome and float in a 40°C water bath containing distilled water.

8. Transfer the sections onto glass slides suitable for immunohistochemistry. Allow the slides to dry overnight and store slides at room temperature until ready for use. Bake for 2 hours at 60 degree celcius. Mounts 3-4 micron formalin fixed paraffin embedded tissues on saline-coted slides.

Immunostain formalin-fixed, paraffin-embedded tissue sections (Step 9-29):

9. Deparaffinize slides in xylene for 2 times, 5 min each.

10. Transfer slides to 100% alcohol, for 2 times, 3 min each, and then transfer once through 95%, 70% and 50% alcohols respectively for 3 min each.

11. Rinse with TBS for 2 times.

12. Perform antigen retrieval to unmask the antigenic epitope. The most commonly used antigen retrieval is a citrate buffer method. Arrange the slides in a staining container. Pour 300 ml of 10 mM citrate buffer, pH 6.0 into the staining container and incubate it at 95-100°C for 10 min (optimal incubation time should be determined by user). Remove the staining container to room temperature and allow the slides to cool for 20 min.

13. Rinse slides with TBS for 2 times

14. Block endogenous peroxidase activity by incubating sections in 3% H2O2 solution in Deionized at room temperature for 10 min to block endogenous peroxidase activity

15. Rinse slides with TBS for 2 times

16. Apply 100 µl primary antibody to the sections on the slides and incubate in a Immunostainner at room temperature for 1 h.

17. Wash the slides with TBS for 2 times.

19. Wash slides with TBS for 2 times

20. Apply 100 µl appropriately HRP conjugates to the sections on the slides and incubate in a Immunostainner at room temperature for 30 min (keep protected from light).

21. Wash slides with TBS for 2 times

22. Apply DAB substrate solution (freshly made just before use): Allow the color development for < 5 min until the desired color intensity is reached. (Caution: DAB is a suspect carcinogen. Handle with care. Wear gloves, lab coat and eye protection.)

24. Wash slides with TBS for 3 times.

25. Counterstain slides by immersing slides in Hematoxylin for 1-2 min.

26. Rinse the slides in running tap water for 2 min.

27. Dehydrate the tissue slides through 4 times of alcohol (95%, 95%, 100% and 100%), 5 min each.

28. Clear the tissue slides in 3 times of xylene and coverslip using DPX mounting solution. The mounted slides can be stored at room temperature permanently.

29. Observe the color of the antibody staining in the tissue sections under microscopy.

ANNEXURE VI

2015 WHO CLASSIFICATION OF TUMOURS OF THE LUNG

- **Epithelial Tumours**

- **Adenocarcinoma**

>Lepidic adenocarcinoma

>Acinar adenocarcinoma

>Papillary adenocarcinoma

>Micropapillary adenocarcinoma

>Solid adenocarcinoma

>Invasive mucinous adenocarcinoma

Mixed invasive mucinous and non-mucinous adenocarcinoma

>Colloid adenocarcinoma

>Fetal adenocarcinoma

>Enteric adenocarcinoma

>Minimally invasive adenocarcinoma

Non-mucinous

Mucinous

>Preinvasive lesions

Atypical adenomatous hyperplasia

Adenocarcinoma in situ

Mucinous

Non-mucinous

- **Squamous cell carcinoma**

  >Keratinizing squamous cell carcinoma

  >Non-keratinizing squamous cell carcinoma

  >Basaloid squamous cell carcinoma

  >Pre-invasive lesion

  Squamous cell carcinoma in situ

- **Neuroendocrine tumours**

  >Small cell carcinoma

  Combined small cell carcinoma

  >Large cell neuroendocrine carcinoma

  Combined large cell neuroendocrine carcinoma

  >Carcinoid tumours

  Typical carcinoid

  Atypical carcinoid

  >Preinvasive lesion

  Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

- **Large cell carcinoma**
- **Adenosquamous carcinoma**
- **Pleomorphic carcinoma**
- **Spindle cell carcinoma**
- **Giant cell carcinoma**
- **Carcinosarcoma**
- **Pulmonary blastoma**

- **Other and unclassified carcinomas**

  >Lymphoepithelioma - like carcinoma

  >NUT carcinoma

- **Salivary gland-type tumours**

  >Mucoepidermoid carcinoma

  >Adenoid cystic carcinoma

  >Epithelial-myoepithelial carcinoma

  >Pleomorphic adenoma

- **Papillomas**

  > Squamous cell papilloma

  Exophytic

  Inverted

  > Glandular papilloma

  >Mixed squamous cell and glandular papilloma

- **Adenomas**

  >Sclerosing pneumocytoma

  >Alveolar adenoma

  >Papillary adenoma

  >Mucinous cystadenoma

  >Mucous gland adenoma

- **Mesenchymal tumours**
  - **Pulmonary hamartoma**
  - **Chondroma**
  - **PEComatous tumours**

    >Lymphangioleiomyomatosis

    >PEComa, benign

    Clear cell tumour

    >PEComa, malignant
  - **Congenital peribronchial myofibroblastic tumour**
  - **Diffuse pulmonary lymphangiomatosis**
  - **Inflammmatory myofibroblastic tumour**
  - **Epithelioid hemangioendothelioma**
  - **Pleuropulmonary blastoma**
  - **Synovial sarcoma**
  - **Pulmonary artery intimal sarcoma**
  - **Pulmonary myxoid sarcoma with EWSR1-CREB1 translocation**
  - **Myoepithelial tumours**

    >Myoepithelioma

    >Myoepithelial carcinoma

- **Lymphohistiocytic tumours**
  - **Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue(MALT Lymphoma)**
  - **Diffuse large B-cell lymphoma**
  - **Lymphomatoid granulomatosis**

- **Intravascular large B-cell lymphoma**
- **Pulmonary langerhans cell histiocytosis**
- **Erdheim-chester disease**

- **Tumours of ectopic origin**
- **Germ cell tumours**

  >Teratoma, mature

  >Teratoma, immature
- **Intrapulmonary thymoma**
- **Melanoma**
- **Meningioma, NOS**

- **Metastatic tumours**

ANNEXURE VII

TNM STAGING (WHO)

T- Primary Tumour

TX Primary tumour cannot be assessed, or tumour proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy

TO No evidence of primary tumour

Tis Carcinoma in situ

T1 Tumour ≤3cm in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus, i.e. not in the main bronchus

T1a Tumour ≤2cm in greatest dimension

T1b Tumour >2cm but ≤3 cm in greatest dimension

T2 Tumour >3cm but ≤7 cm or tumour with any of the following features (T2 tumours with these features are classified T2a if ≤5 cm or if size cannot be determined and T2b if >5 cm but ≤7 cm).

- Involves main bronchus, ≥2cm distal to carina
- Invades visceral pleura
- Associated with atelectasis or obstruction pneumonitis that extends to the hilar region but does not involve the entire lung

T2a Tumour >3cm but ≤5 cm in greatest dimension

T2b Tumour >5 cm but ≤7cm in greatest dimension

T3 Tumour >7cm or one that directly invades any of the following: chest wall(including superior sulcus tumours), diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium; or tumour in the man bronchus <2cm distal to the carina but without involvement of the carina; or associated atelectasis or obstructive

pneumonitis of the entire lung ; or separate tumour nodule(s) in the same lobe as the primary

T4 Tumour of any size that invades any of the following mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, oesophagus, vertebra body, carina; separate tumour nodule(s) in a different ipsilateral lobe to that of the primary

N-Regional Lymph node

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension

N2 Metastasis in ipsilateral mediastinal and/or subcarinal lymph nodes

N3 Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

M-Distant Metastasis

M0 No distant metastasis

M1 Distant metastasis

M1a Separate tumour nodule(s) in a contralateral lobe,tumour with pleural nodules or malignant pleural or pericardial effusion

M1b Distant Metastasis

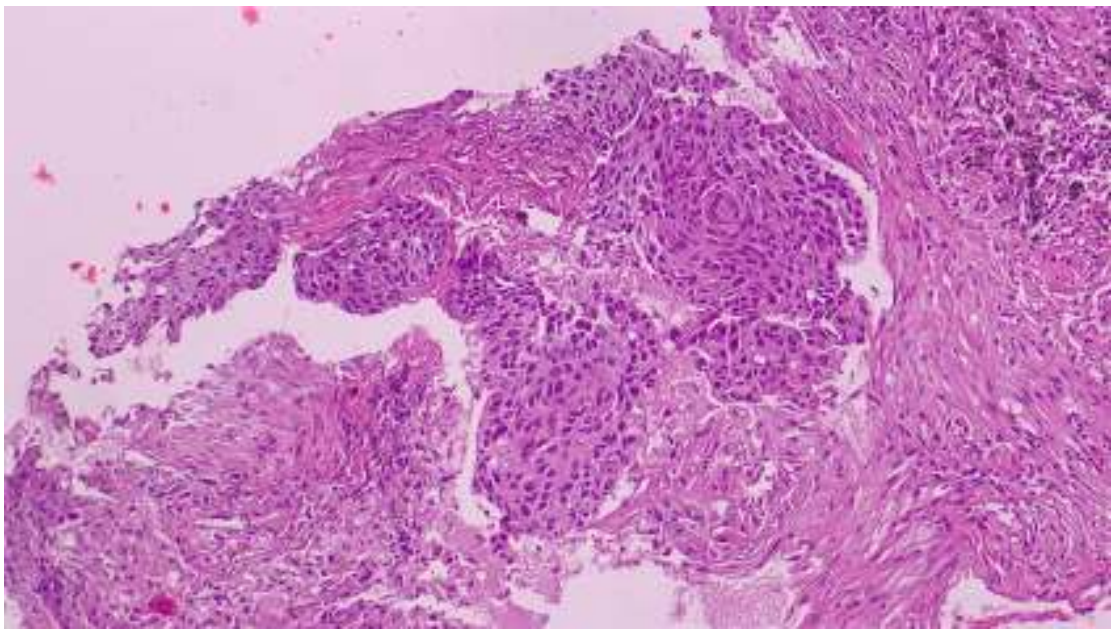
Stage grouping

Occult carcinoma	Tx	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1a	N0	M0
	T1b	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T1a	N1	M0
	T1b	N1	M0
	T2a	N1	M0
	T2b	N0	M0
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
	T4	N0	M0
	T4	N1	M0
Stage IIIB	T4	N2	M0
	Any T	N3	M0
Stage IV	Any T	Any N	M1a
	Any T	Any N	M1b

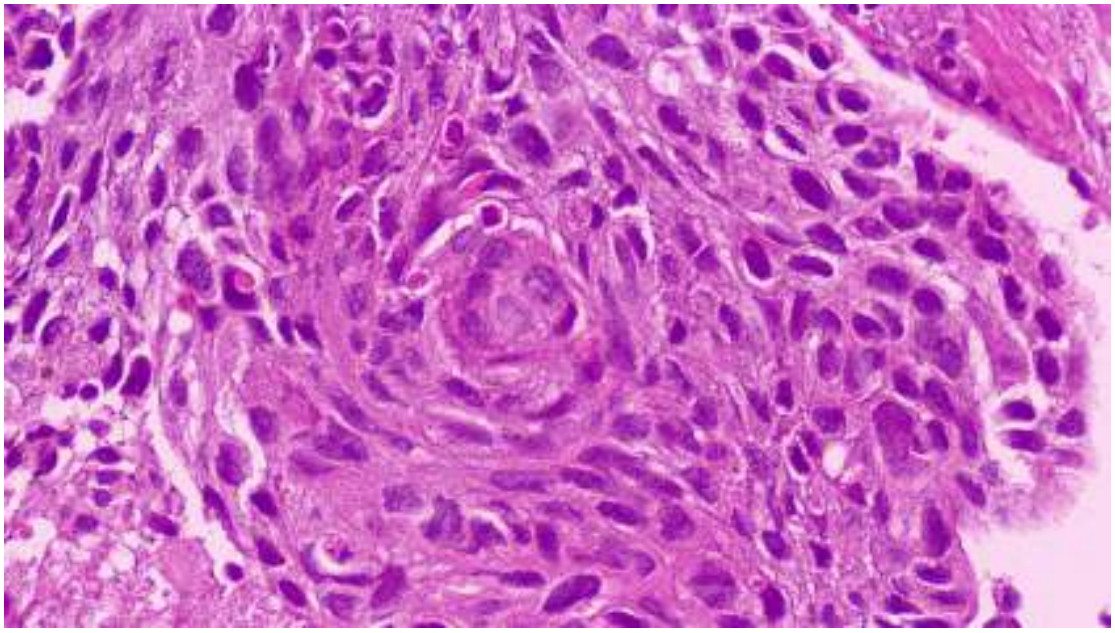
ANNEXURE VIII

PHOTOMICROGRAPHS

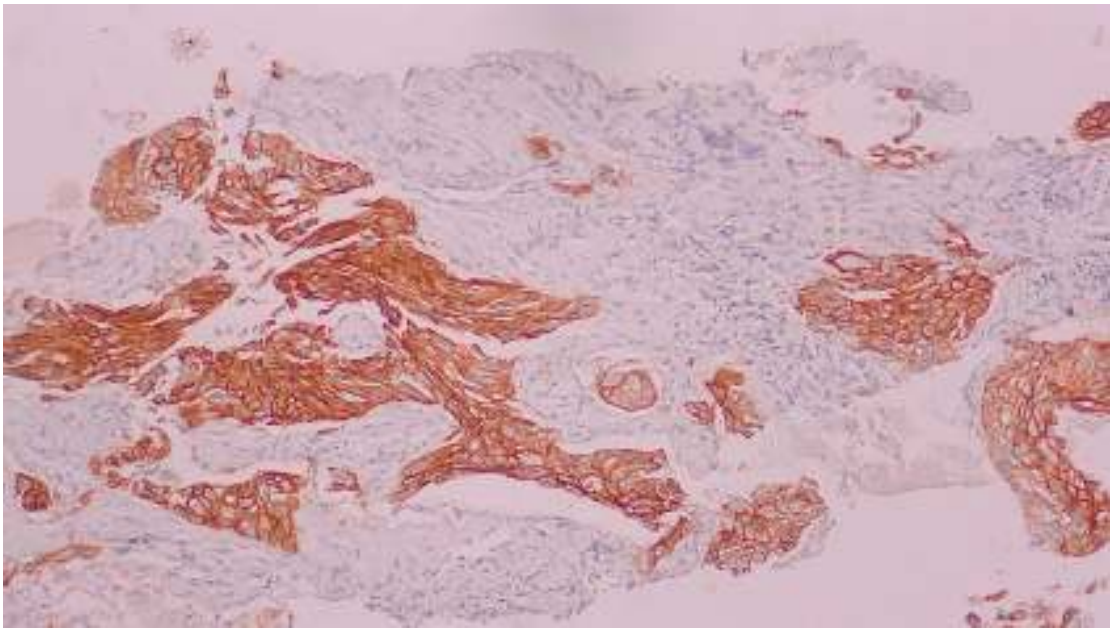
Case 588/20:



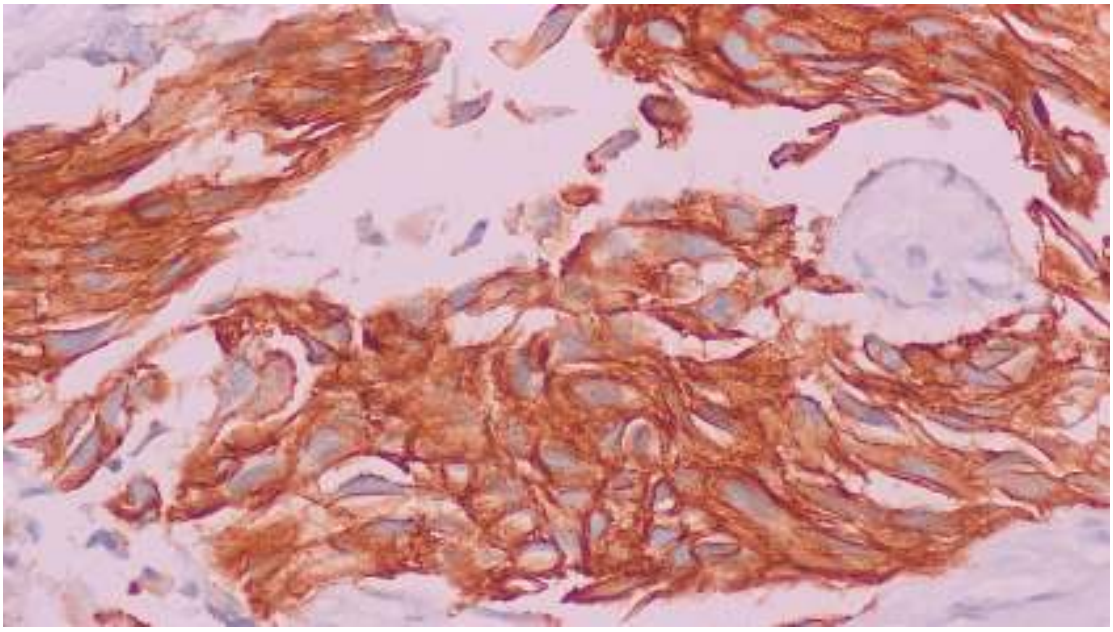
Photomicrograph 1 : Squamous cell carcinoma; 10x; H&E



Photomicrograph 2 : Squamous cell carcinoma; 40x; H&E

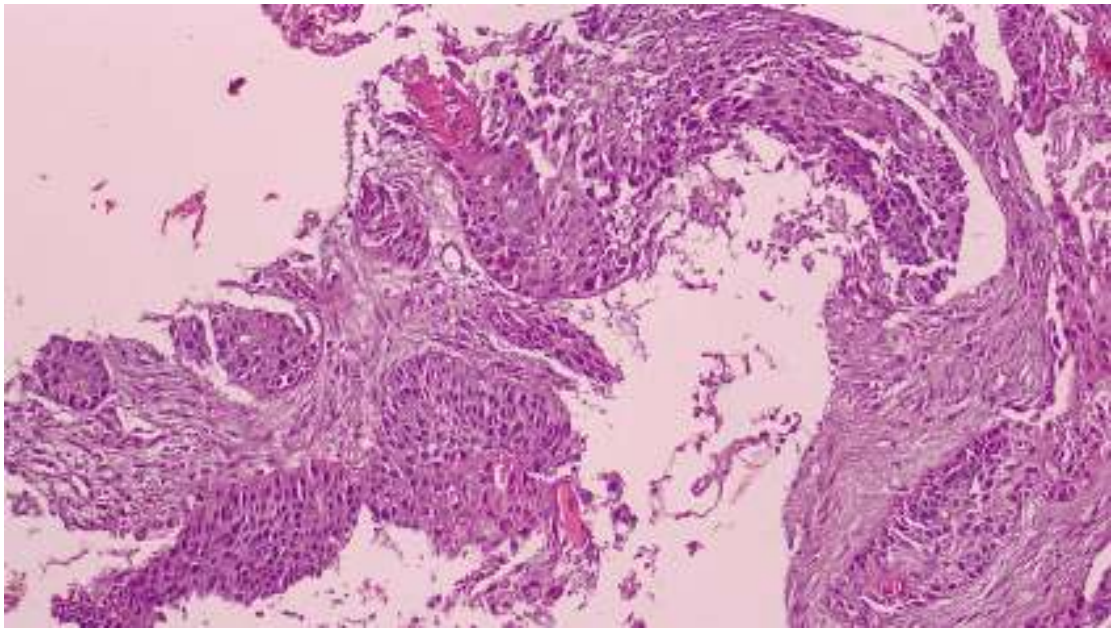


Photomicrograph 3 : Squamous cell carcinoma; 10x; EGFR IHC staining intensity=3+ (strong staining)

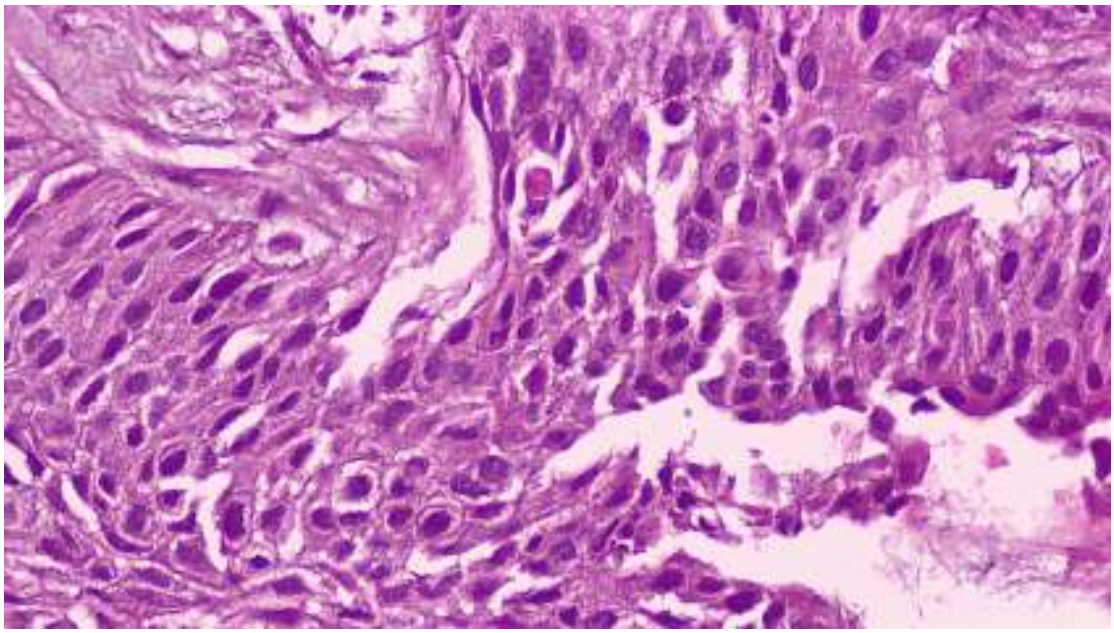


Photomicrograph 4 : Squamous cell carcinoma; 40x; EGFR IHC staining intensity=3+ (strong staining)

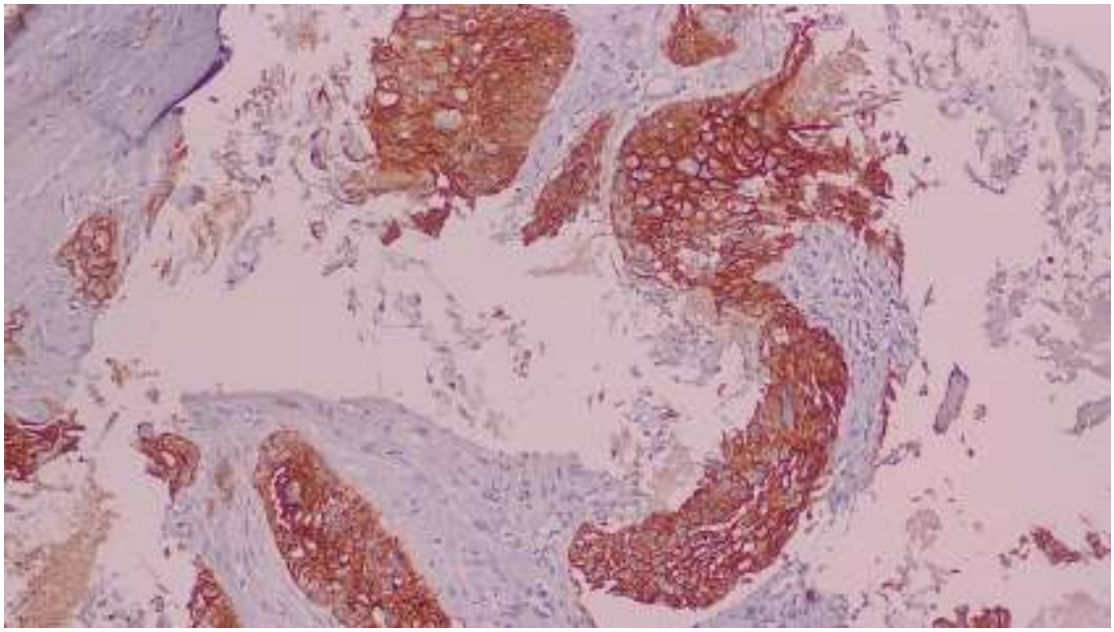
Case 4621/19:



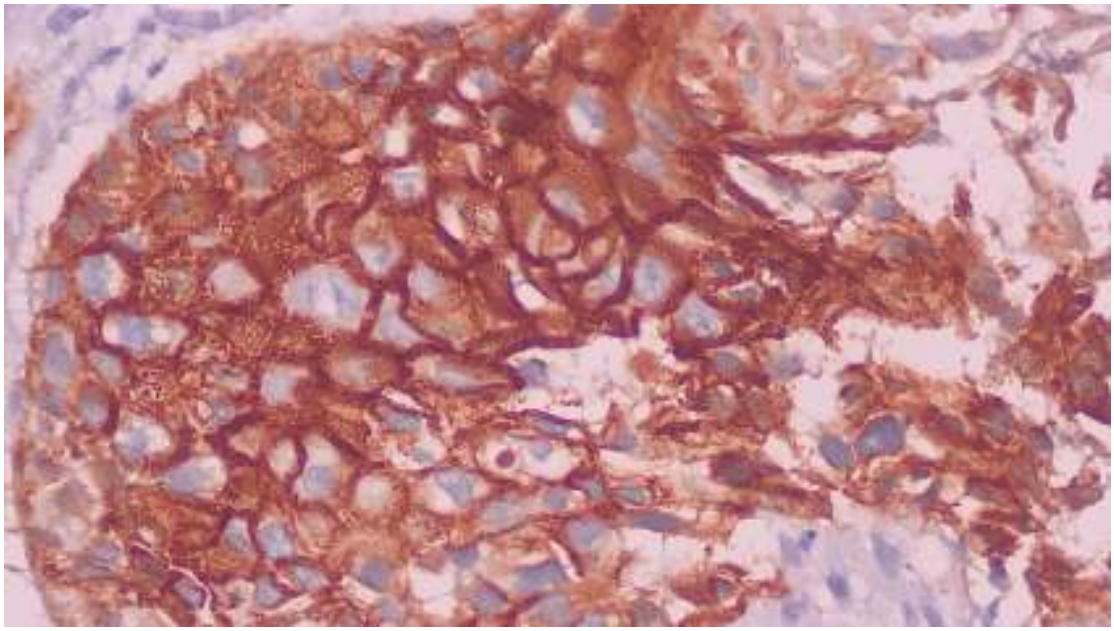
Photomicrograph 5 : Squamous cell carcinoma; 10x; H&E



Photomicrograph 6 : Squamous cell carcinoma; 40x; H&E

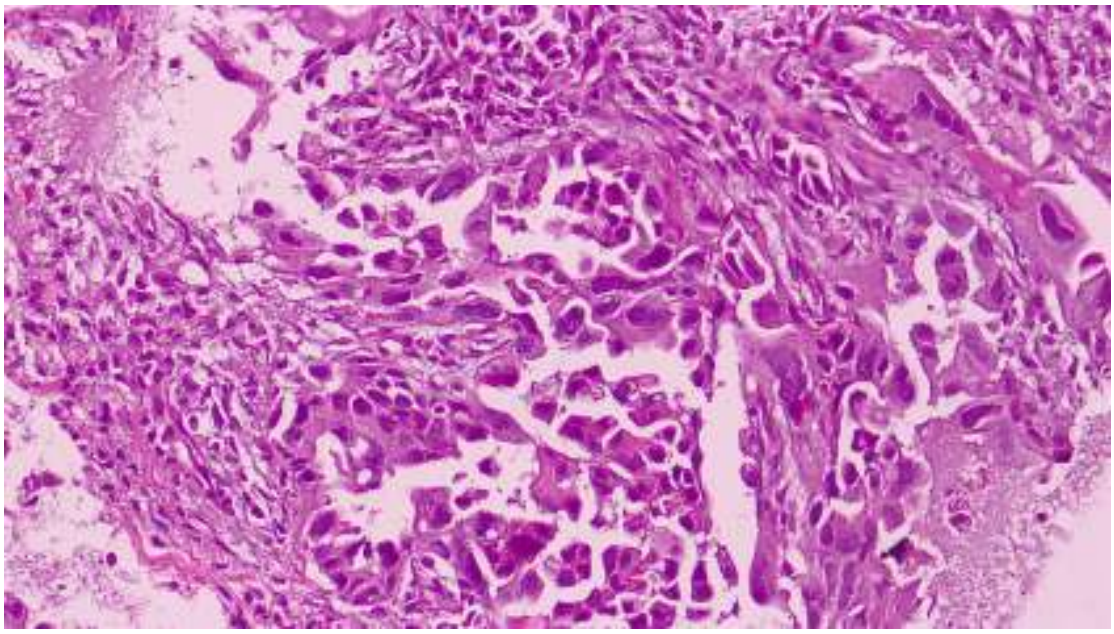


Photomicrograph 7 : Squamous cell carcinoma; 10x; EGFR IHC staining intensity= 2+ (intermediate staining)

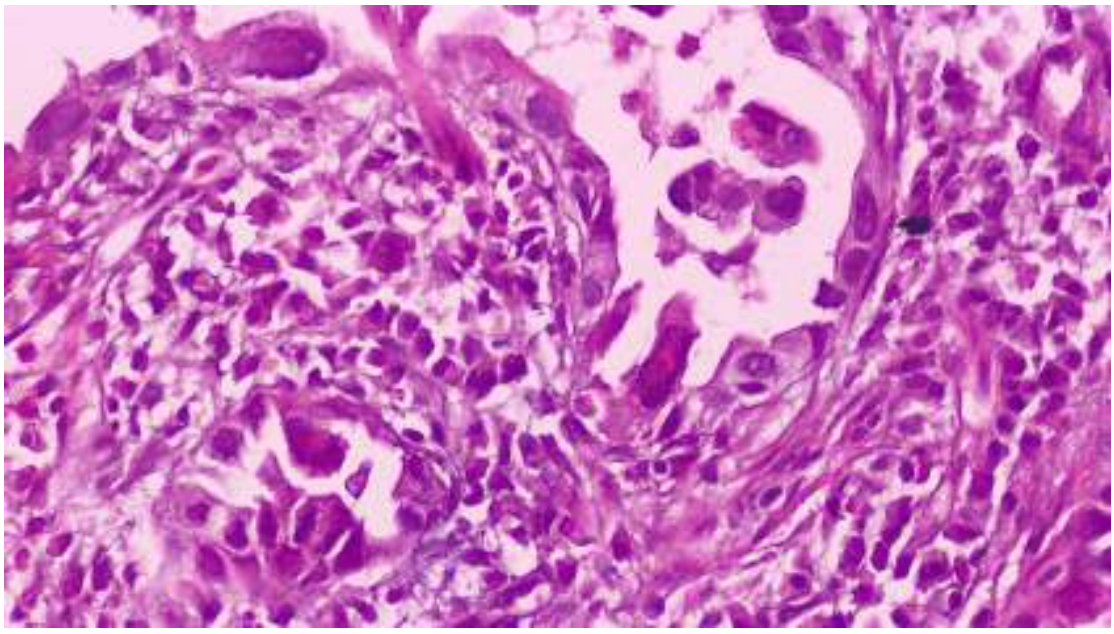


Photomicrograph 8 : Squamous cell carcinoma; 40x; EGFR IHC staining intensity= 2+ (intermediate staining)

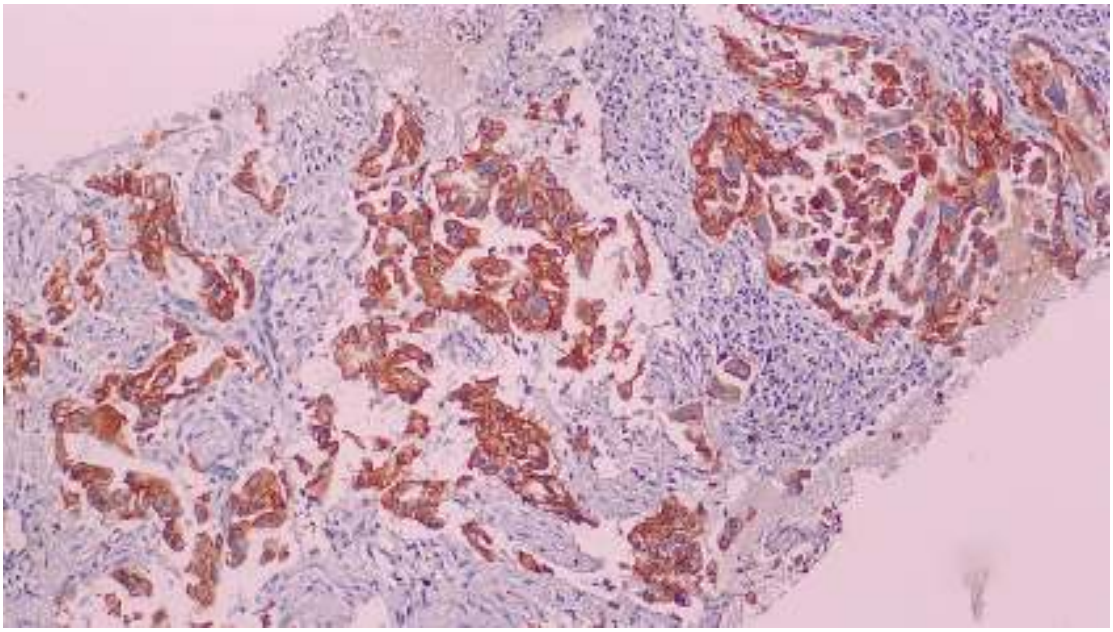
Case 1333/20:



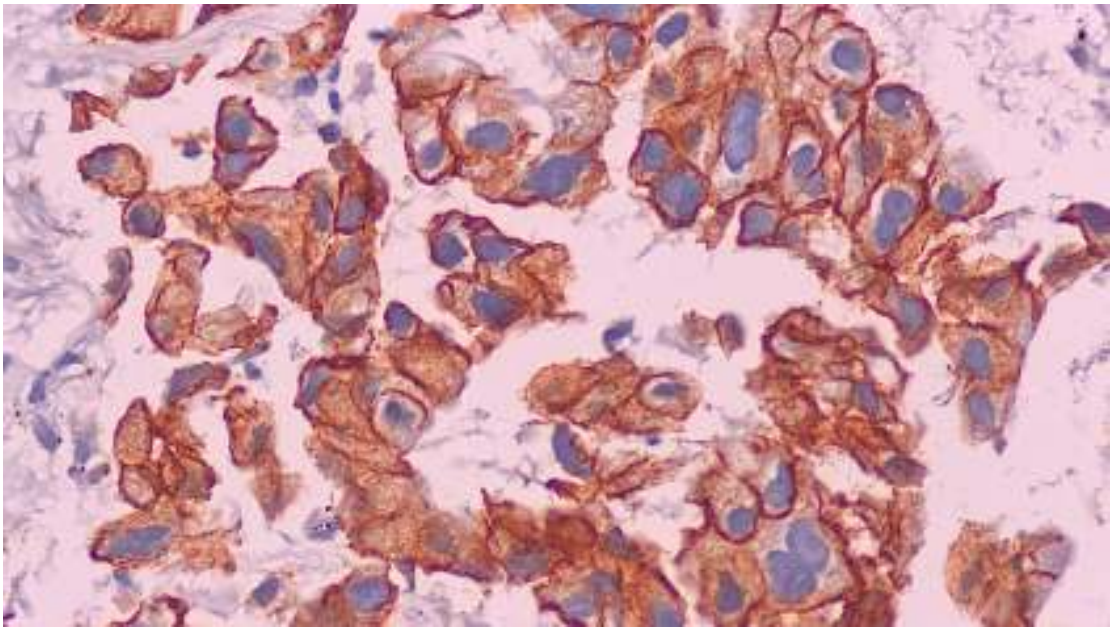
Photomicrograph 9 : Adenocarcinoma; 20x; H&E



Photomicrograph 10 : Adenocarcinoma; 40x; H&E

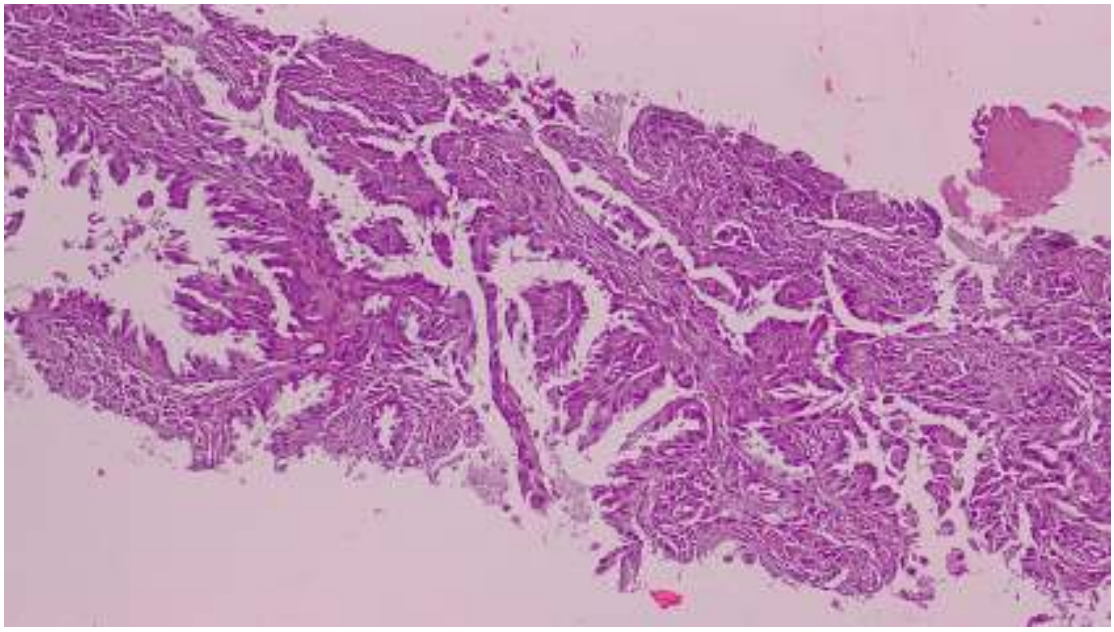


Photomicrograph 11 : Adenocarcinoma; 10x; EGFR IHC staining intensity=3+ (strong staining)

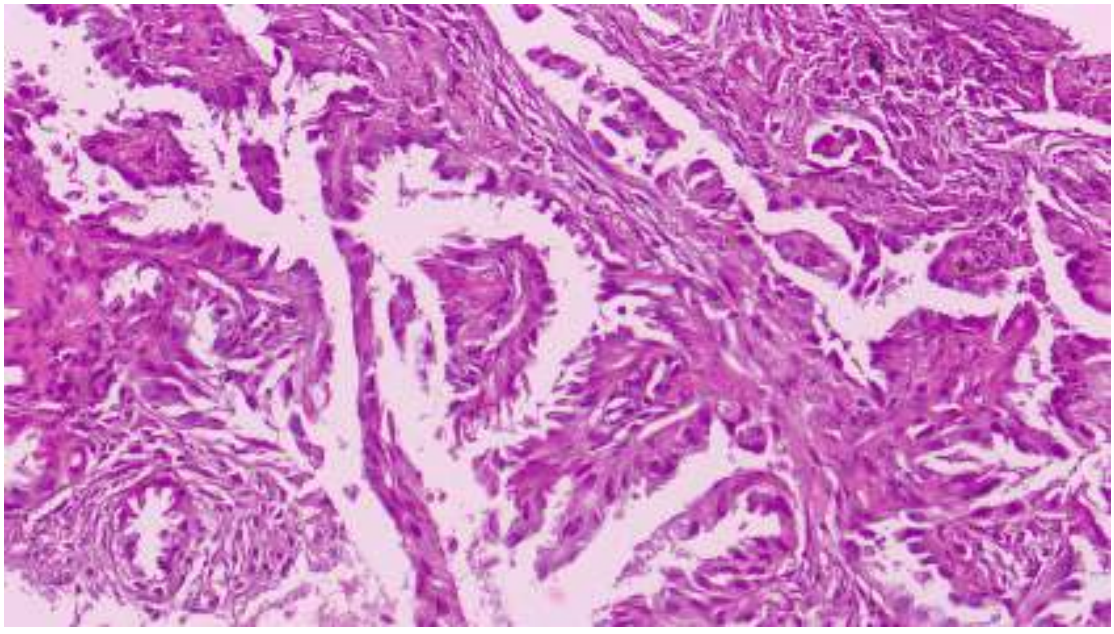


Photomicrograph 12 : Adenocarcinoma; 40x; EGFR IHC staining intensity=3+ (strong staining)

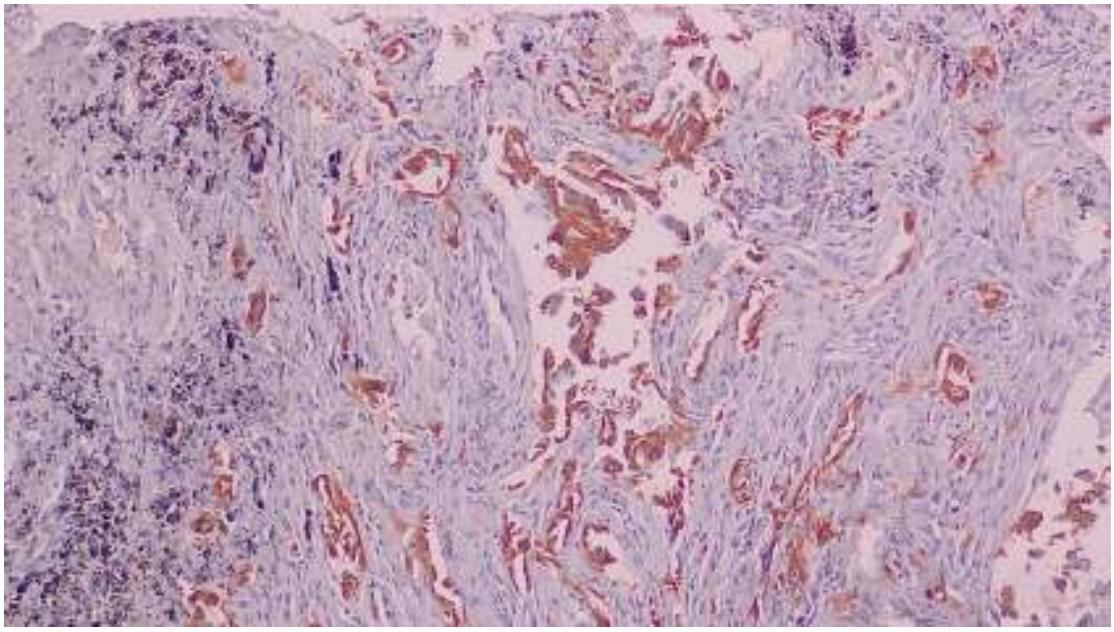
Case 1073/19 :



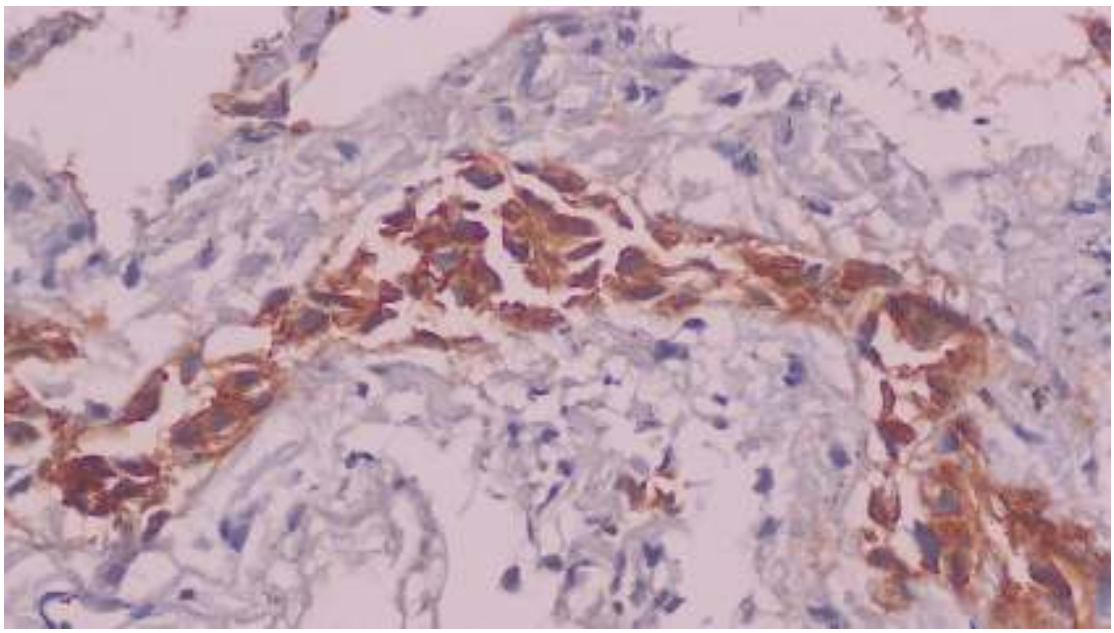
Photomicrograph 13 : Adenocarcinoma; 10x; H&E



Photomicrograph 14 : Adenocarcinoma; 20x; H&E

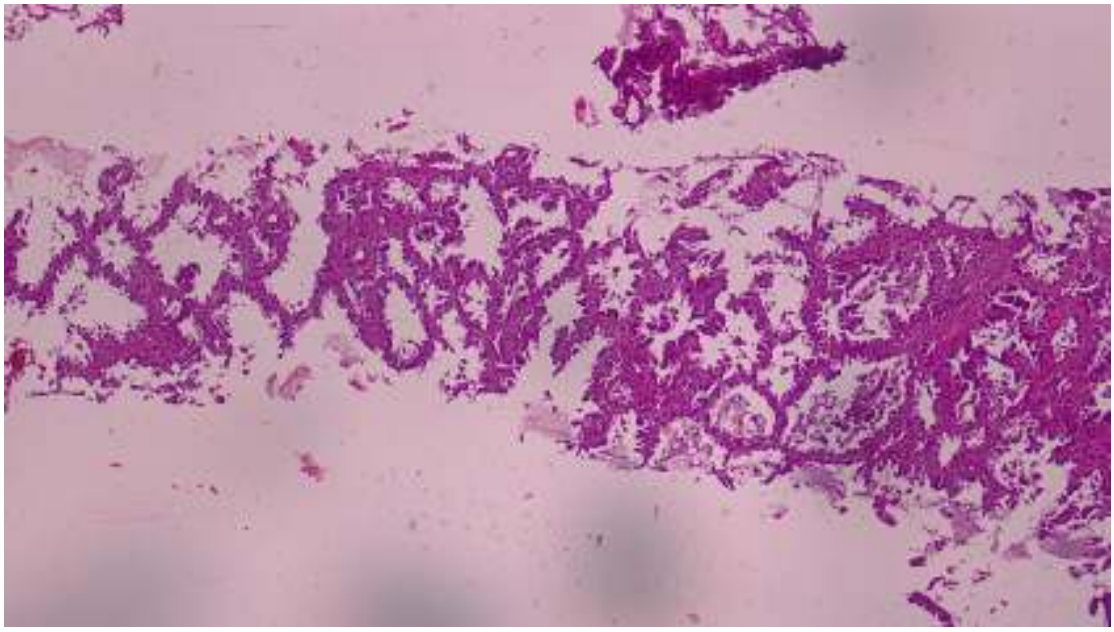


Photomicrograph 15 : Adenocarcinoma; 10x; EGFR IHC staining intensity=3+ (strong staining)

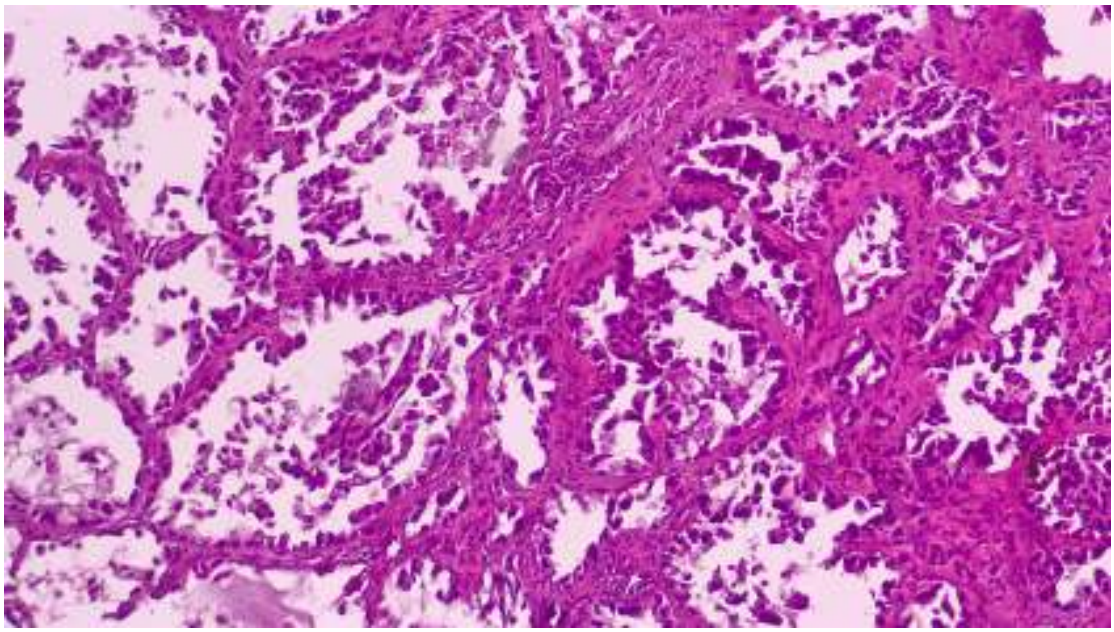


Photomicrograph 16 : Adenocarcinoma; 40x; EGFR IHC staining intensity=3+ (strong staining)

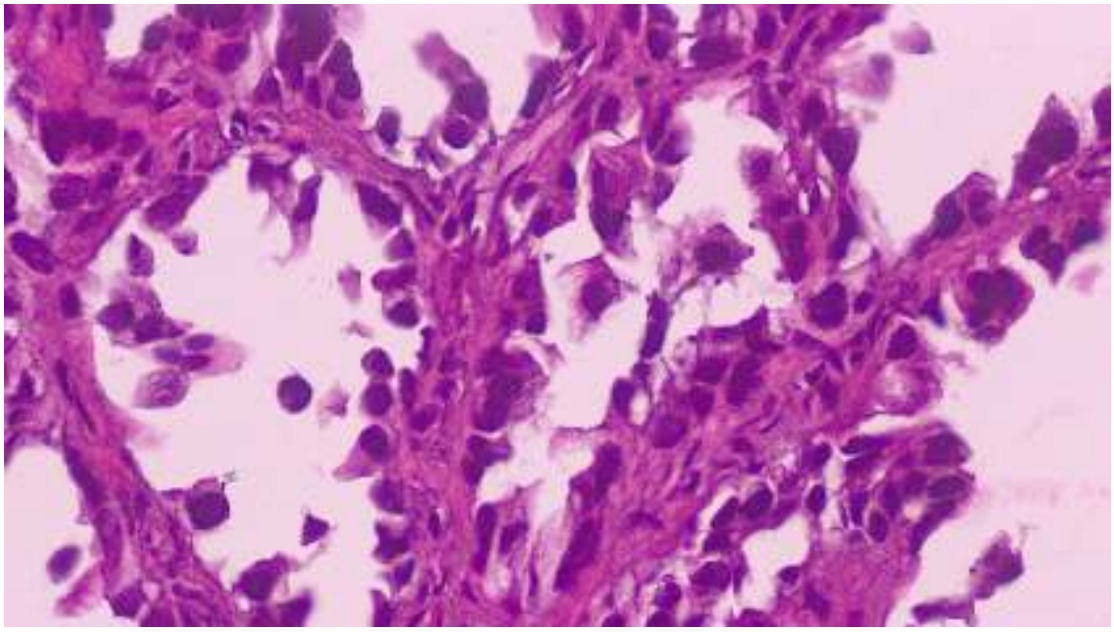
Case 4322/19:



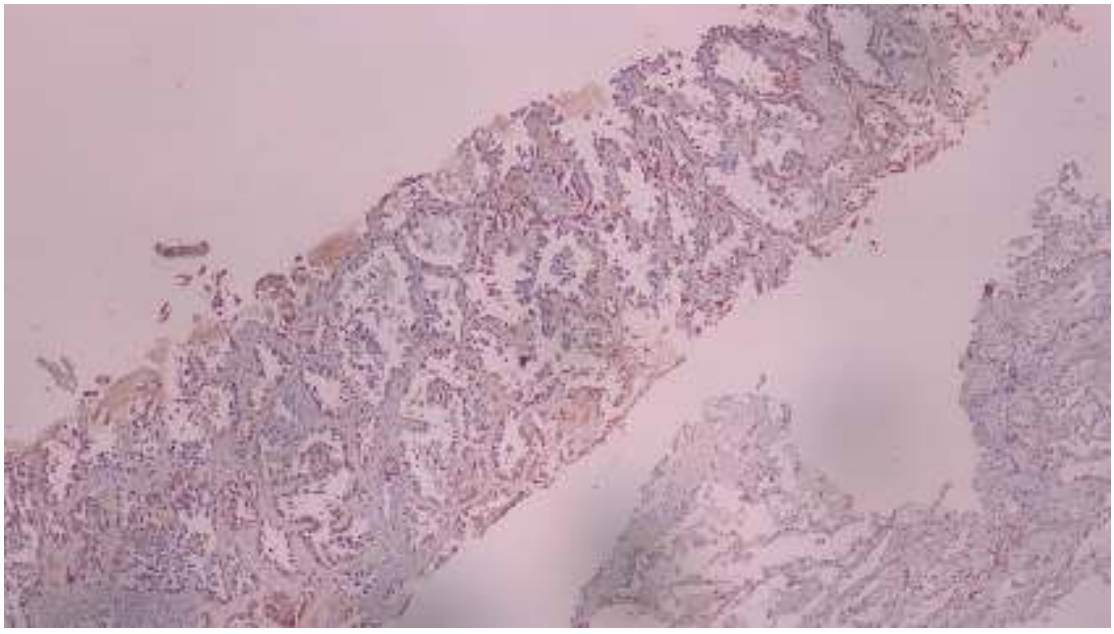
Photomicrograph 17 : Lepidic Adenocarcinoma; 4x; H&E



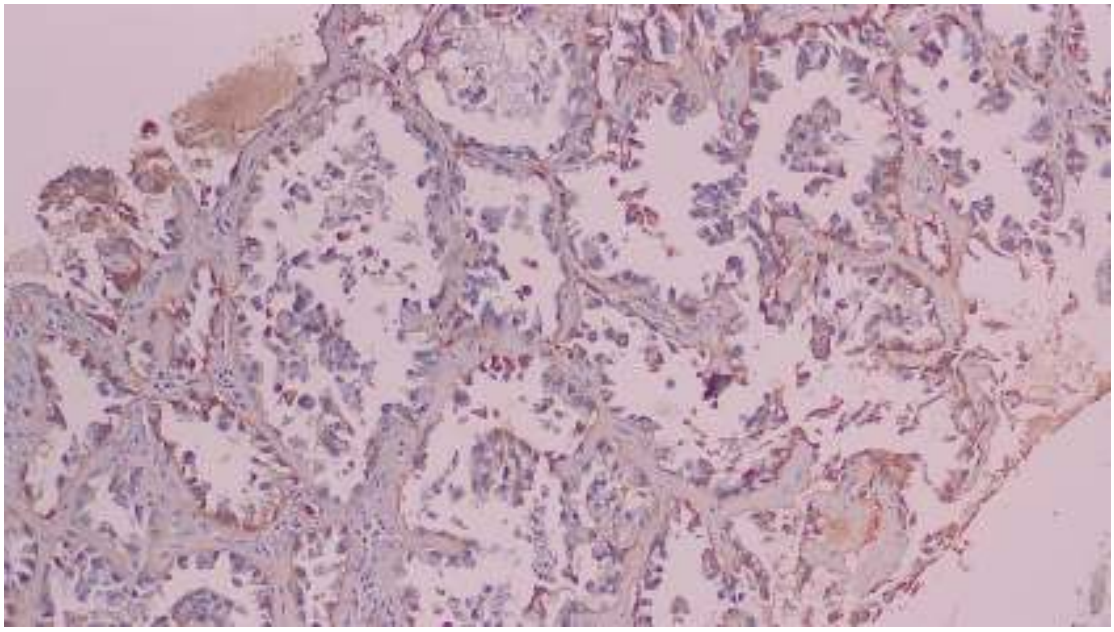
Photomicrograph 18 : Lepidic Adenocarcinoma; 10x; H&E



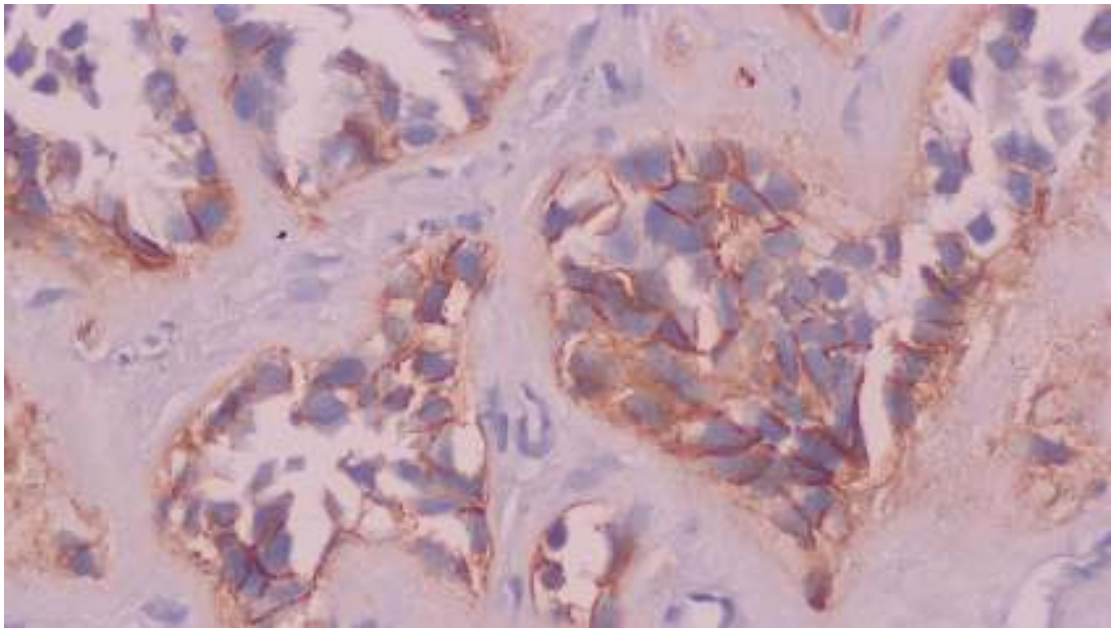
Photomicrograph 19 : Lepidic Adenocarcinoma; 40x; H&E



Photomicrograph 20 : Lepidic Adenocarcinoma; 4x; EGFR IHC staining intensity= 2+ (intermediate staining)

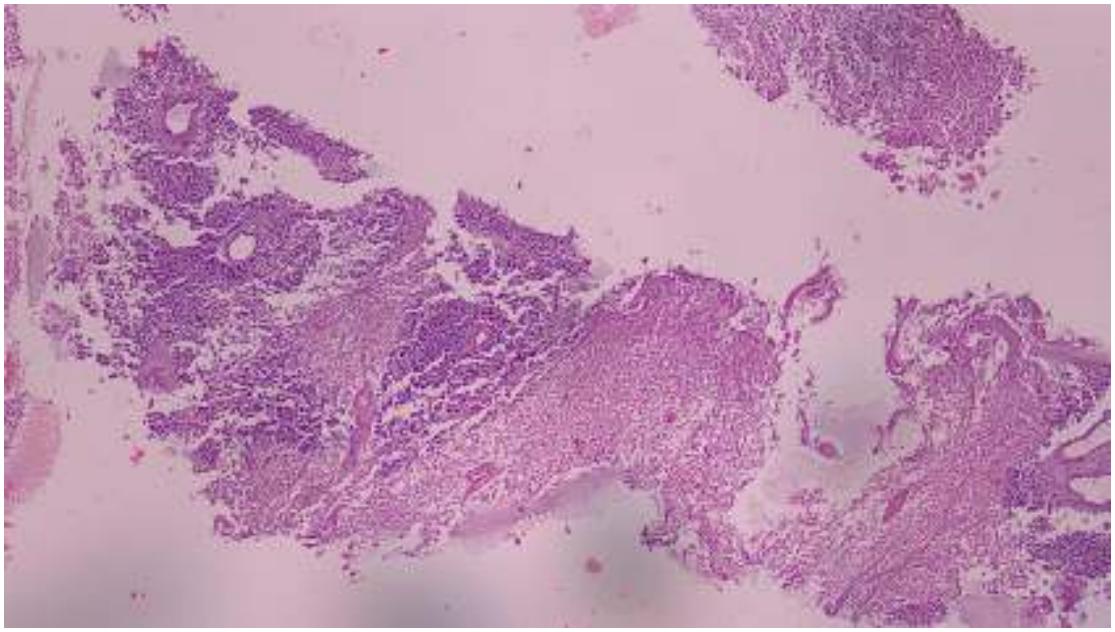


Photomicrograph 21 : Lepidic Adenocarcinoma; 10x; EGFR IHC staining intensity= 2+ (intermediate staining)

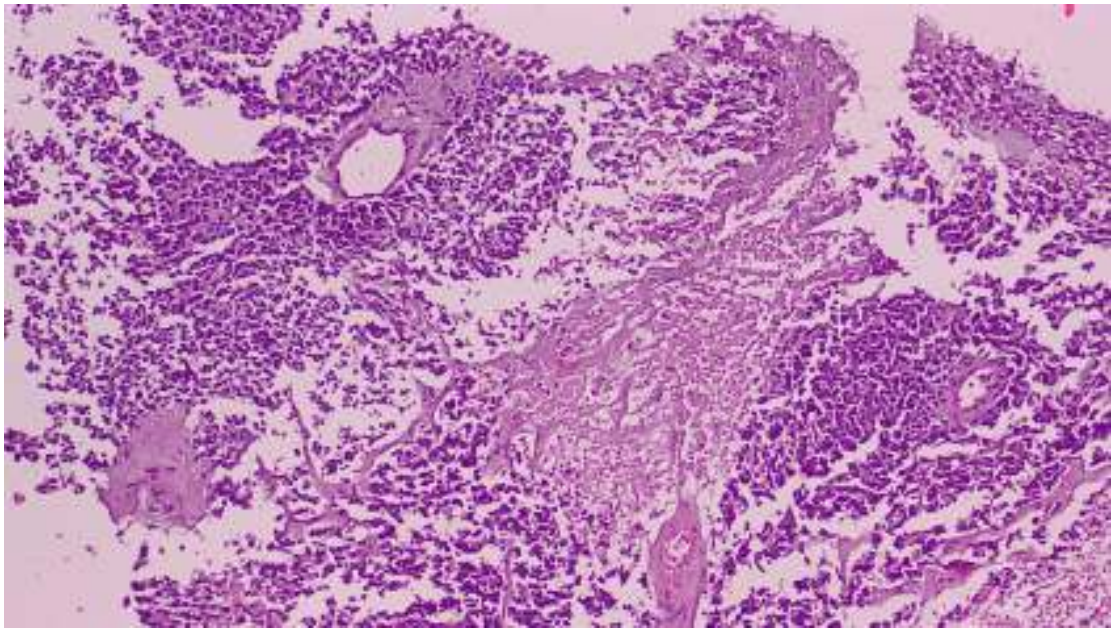


Photomicrograph 22 : Lepidic Adenocarcinoma; 40x; EGFR IHC staining intensity= 2+ (intermediate staining)

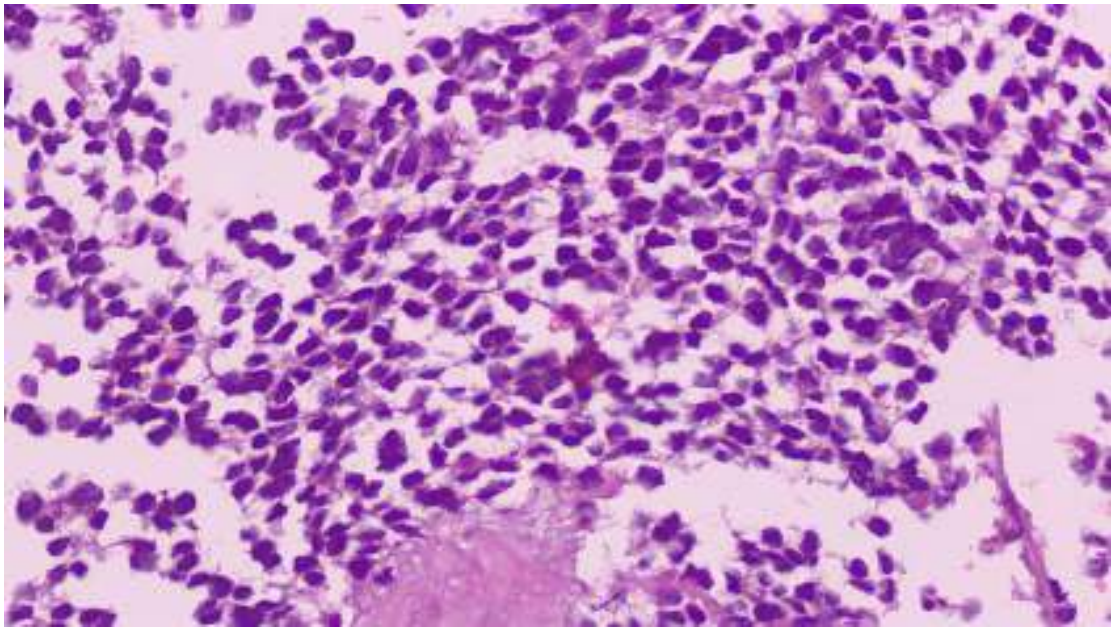
Case 1910/19:



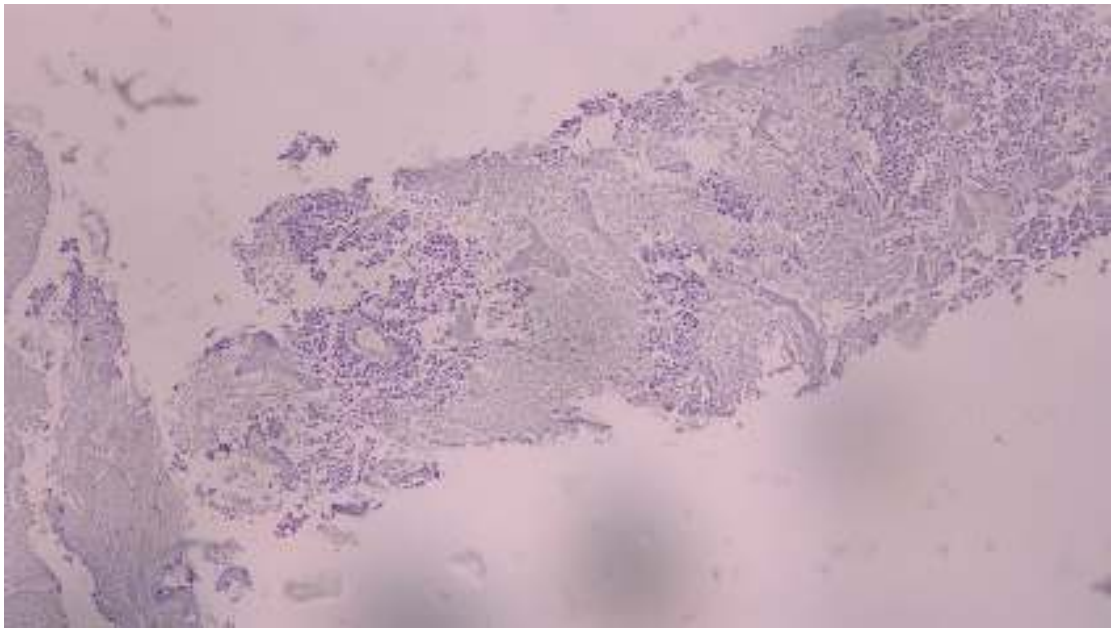
Photomicrograph 23 : Small cell carcinoma; 4x; H&E



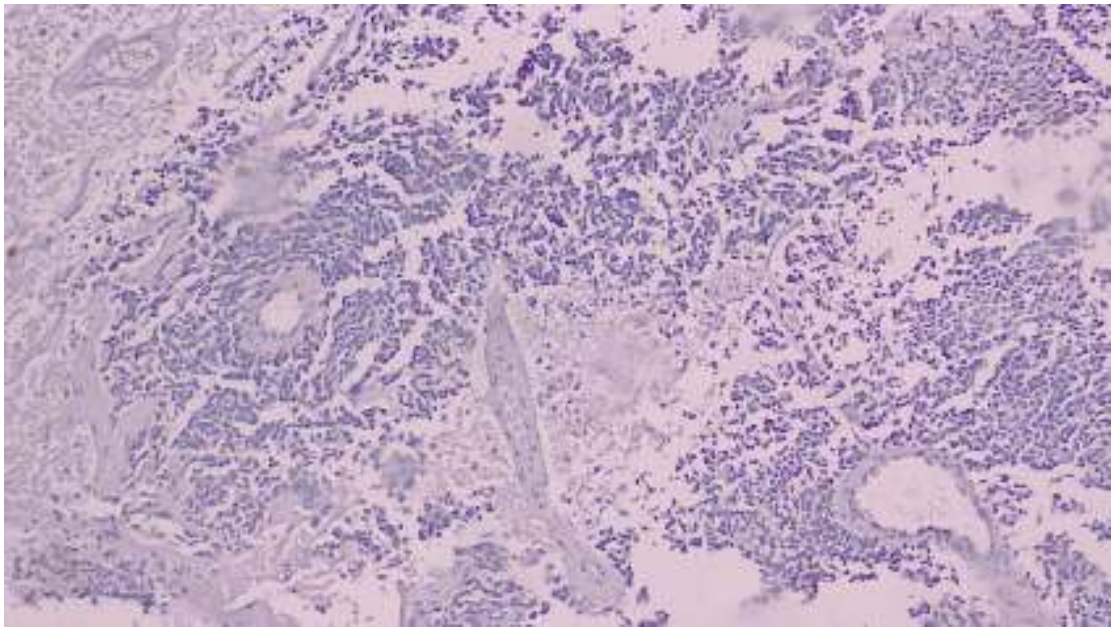
Photomicrograph 24 : Small cell carcinoma; 10x; H&E



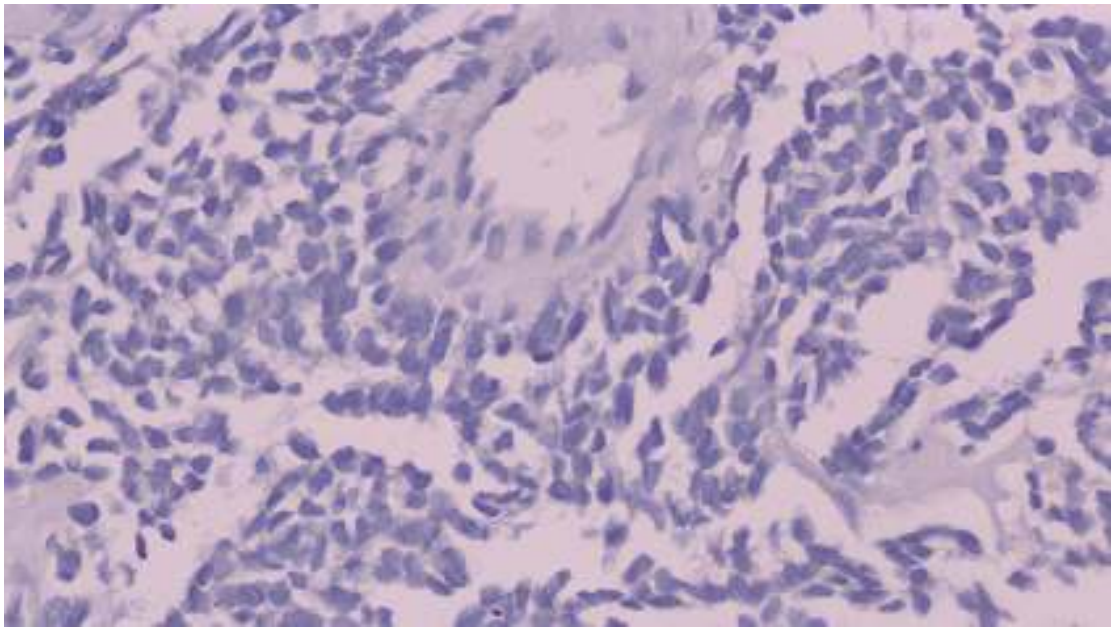
Photomicrograph 25 : Small cell carcinoma; 40x; H&E



Photomicrograph 26 : Small cell carcinoma; 4x; EGFR IHC staining intensity= 0

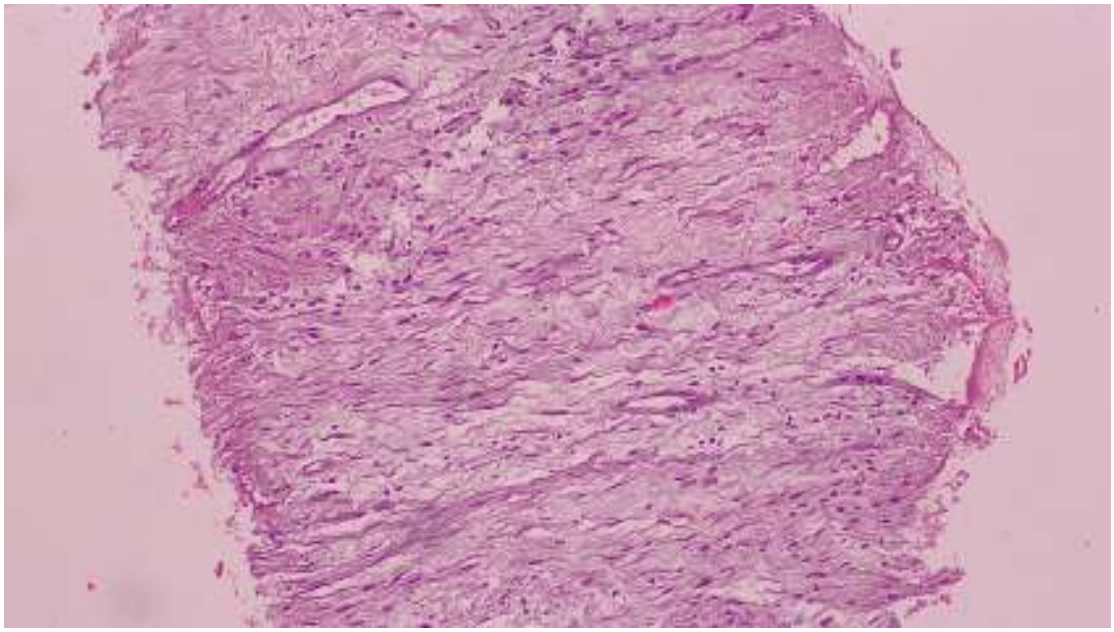


Photomicrograph 27 : Small cell carcinoma; 10x; EGFR IHC staining intensity= 0

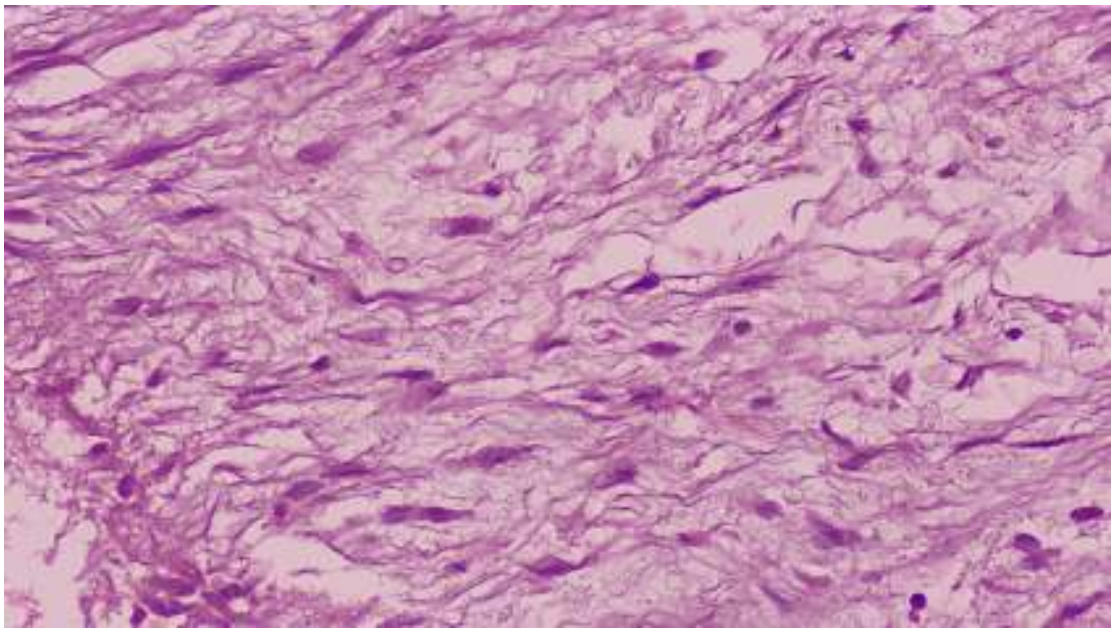


Photomicrograph 28 : Small cell carcinoma; 40x; EGFR IHC staining intensity= 0

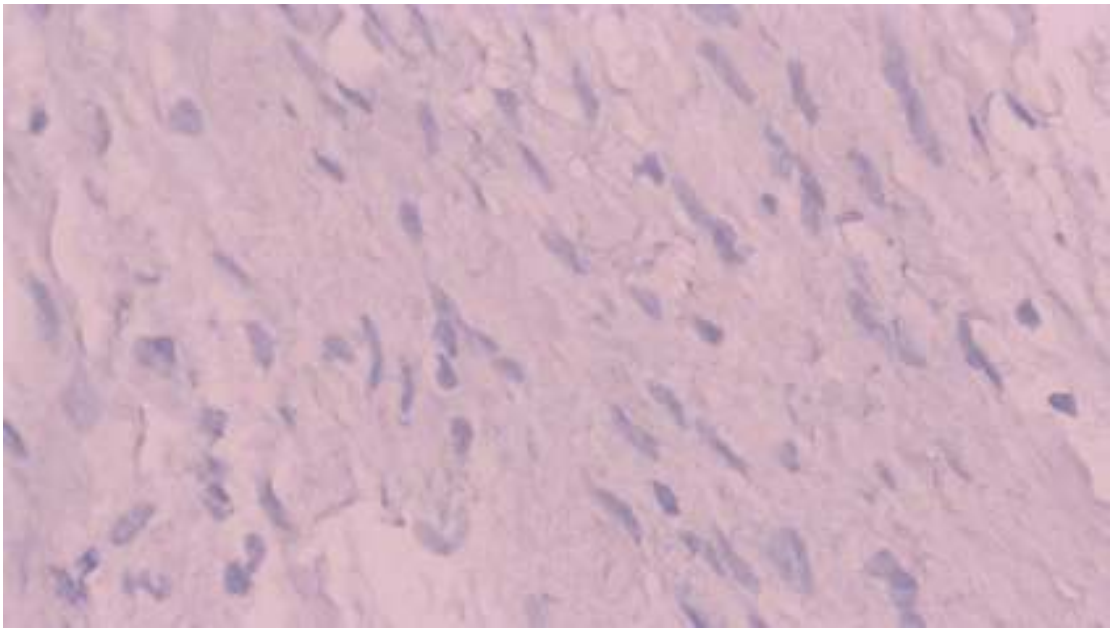
Case 4334/19:



Photomicrograph 29 : Spindle cell neoplasm; 10x; H&E

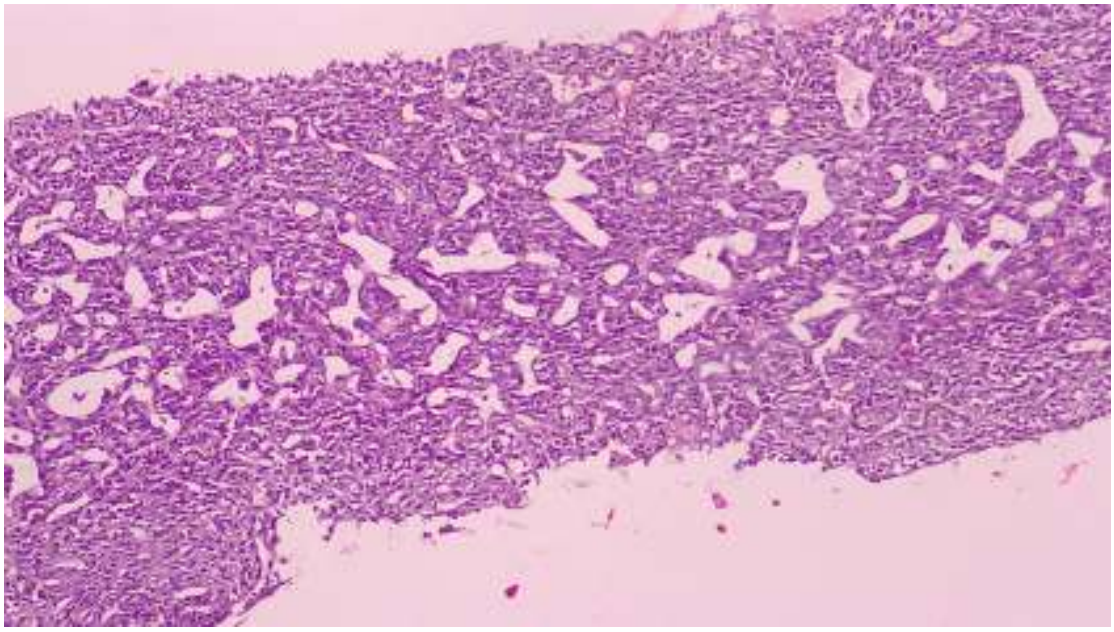


Photomicrograph 30 : Spindle cell neoplasm; 40x; H&E

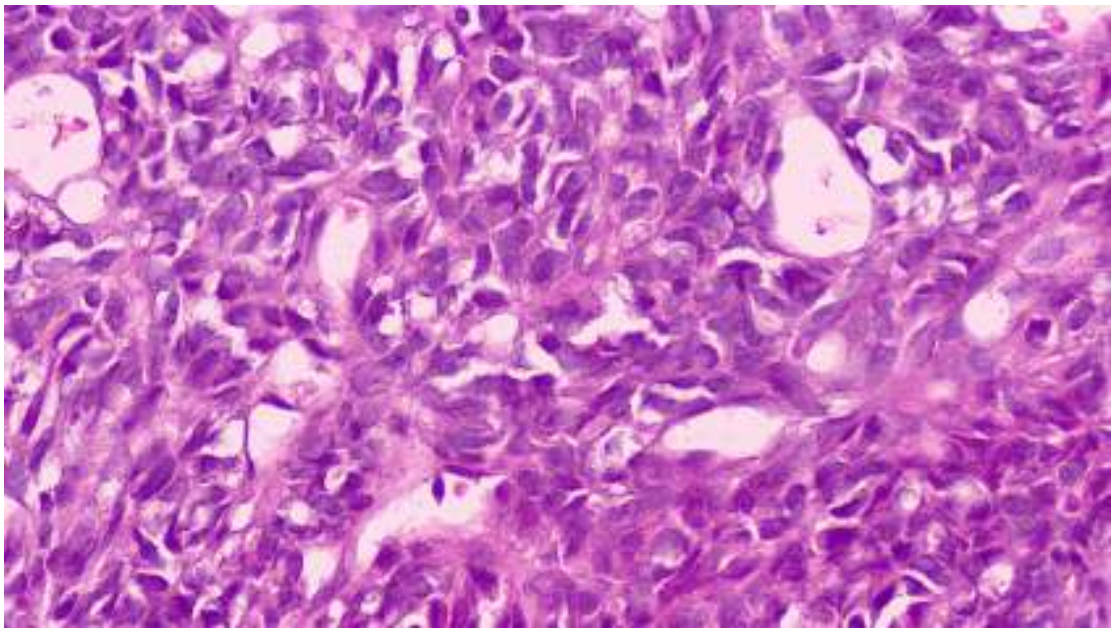


Photomicrograph 31: Spindle cell neoplasm; 40x; EGFR IHC staining intensity=0

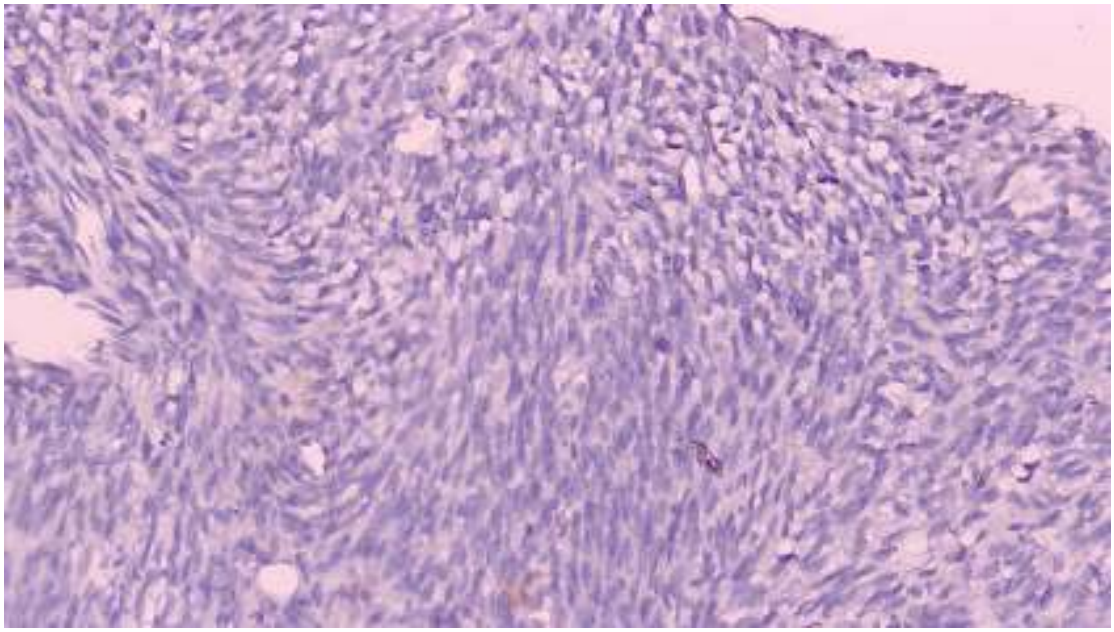
Case 4327/19:



Photomicrograph 32 : Sarcoma lung; 10x; H&E



Photomicrograph 33 : Sarcoma lung; 40x; H&E



Photomicrograph 34: Sarcoma lung; 40x; EGFR IHC staining intensity=0

ANNEXURE X

MASTERCHART

SR NO	biopsy no	AGE(years)	Sex	Site of tumour	Histological type	EGFR Staining intensity	H-Score	Smoking status	Cough	dyspnoea	loss of appetite	Weight loss	Diet	hemoptysis	family history
1	185/19	60	2	2	1	2	1	2	2	1	1	2	1	2	2
2	332/19	54	1	1	4	3	1	1	1	1	1	1	1	1	2
3	350/19	68	2	1	1	2	1	2	2	1	1	1	1	2	1
4	698/19	52	1	1	2	2	1	1	1	1	1	1	2	1	2
5	1073/19	70	1	1	1	3	1	1	1	1	1	1	2	1	2
6	1313/19	55	1	2	1	1	2	1	1	1	2	2	1	2	2
7	1363/19	70	2	4	2	3	1	2	1	1	1	1	2	1	1
8	1442/19	60	1	3	1	0	2	1	1	2	2	2	2	2	2
9	1443/19	80	1	2	1	2	1	1	1	1	1	1	1	1	2
10	1532/19	71	1	4	2	3	1	1	1	1	1	1	2	1	2
11	1910/19	22	1	2	6	0	2	1	2	2	2	2	2	2	2
12	2008/19	63	1	2	1	3	1	1	1	1	1	1	2	2	1
13	2210/19	76	1	1	1	3	1	1	1	1	1	1	2	1	2
14	2535/19	59	1	1	1	2	2	1	1	2	2	2	1	2	2
15	2621/19	58	1	1	1	2	1	1	1	2	2	1	1	2	2
16	2748/19	60	1	1	1	2	1	1	1	2	1	1	2	2	2
17	3220/19	50	2	4	2	3	1	2	1	1	1	1	2	1	1
18	3751/19	55	1	1	1	1	2	2	1	2	2	2	1	2	2

19	4240/19	31	2	1	1	3	1	2	1	2	1	1	1	2	1
20	4322/19	55	2	4	3	2	1	2	1	1	1	1	2	2	1
21	4327/19	40	1	2	5	0	2	1	2	2	2	2	2	2	2
22	4334/19	46	1	1	7	0	2	1	1	2	2	2	1	2	2
23	4405/19	53	1	1	1	3	1	1	1	2	2	1	1	2	2
24	4513/19	62	2	1	2	2	1	2	1	1	1	2	1	1	2
25	4590/19	68	1	1	2	3	1	1	1	1	1	1	2	1	2
26	4621/19	80	1	2	2	2	1	1	1	1	1	1	1	1	2
27	72/20	51	1	1	2	3	1	1	1	1	2	1	2	1	2
28	131/20	69	1	1	2	2	1	1	1	1	1	1	1	1	2
29	588/20	68	1	4	2	3	1	1	1	1	1	1	2	1	2
30	678/20	56	2	1	1	2	1	2	1	2	1	1	1	2	2
31	1027/20	21	2	3	7	0	2	2	1	2	2	2	1	2	2
32	1028/20	60	2	2	1	3	1	2	1	2	2	1	2	2	2
33	1074/20	86	2	3	2	1	2	2	1	1	2	2	1	1	2
34	1170/20	45	1	1	1	3	1	1	1	2	1	1	2	2	2
35	1333/20	64	1	3	1	3	1	1	1	1	1	1	2	2	2
36	1850/20	62	1	2	1	2	1	1	1	1	1	1	2	2	2
37	1879/20	58	2	4	1	1	2	2	1	2	2	2	1	2	2
38	2015/20	65	2	1	1	0	2	2	2	1	2	2	2	1	2
39	2254/20	74	1	3	2	3	1	1	1	1	1	1	1	1	2
40	2448/20	59	1	1	2	2	1	1	1	1	1	1	2	1	2

ANNEXURE IX

KEY TO MASTER CHART

Sex- 1-Male

2-Female

Site of tumour : 1- Right upper lobe

2- Right lower lobe

3- Left upper lobe

4-Left lower lobe

Type of carcinoma - 1=Adenocarcinoma

2=Squamous cell carcinoma

3=Adenocarcinoma-Lepidic variant

4=Squamous cell carcinoma-keratinizing variant

5=Sarcoma

6= Small cell carcinoma

7=spindle cell neoplasm

EGFR expression

0=Negative

1= 1+(Weak positive)

2= 2+ (Intermediate)

3= 3+ (Strong positive)

H-score : 1- =200 or >200

2- < 200

Smoking status-

1: Yes

2: No

Cough - 1-present

2-Absent

Dyspnoea- 1-present

2-absent

Loss of appetite- 1-present

2-absent

Weight loss- 1-present

2-absent

Diet - 1-vegeterian

2-Mixed

Hemoptysis- 1- present

2-absent

Family history- 1-present

2-absent